UNITED STATES CIVIL DEFENSE

BLOOD AND BLOOD DERIVATIVES PROGRAM



FEDERAL CIVIL DEFENSE ADMINISTRATION
(Technical Manual)

United States Government Printing Office · April 1952
(Reprinted June 1955)

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. Price 40 cents

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ACKNOWLEDGMENTS

This manual has been developed in cooperation with the American Medical Association, American Hospital Association, American Association of Blood Banks, Association of State and Territorial Health Officers, American National Red Cross, the U S Public Health Service and the Department of Defense

The recommendations in this manual have the approval of the National Research Council and the Office of Defense Mobilization

Further acknowledgment is made of the following valuable contributors National Institutes of Health, for use of the selected *Minimum Requirements* (supp A), American National Red Cross, for their *Central Supply Room Manual* (supp D), and the National Research Council, for their recommendations in The Place of Saline in Treatment of Injuries With Shock (supp E)

BLOOD AND BLOOD DERIVATIVES PROGRAM FOR CIVIL DEFENSE

Scope of the Program

1.1 The Federal Civil Defense Administration is responsible for assuring the provision of adequate supplies of whole blood, blood plasma, and plasma expanders for the treatment of civilian casualties resulting from enemy attack. To accomplish this a Nation wide blood program must be developed to provide adequate quantities of plasma, plasma expanders and equipment for collecting and administering whole blood for Federal, State, and local reserves of medical supplies. Above all, the active cooperation and assistance of all blood banks and blood donor facilities are needed. In addition, such a Nation wide blood program must have the active cooperation of the American National Red Cross, the American Medical Association, the American Association of Blood Banks, the American Society of Clinical Pathologists, the American Hospital Association, and other professional organizations concerned, as well as the Department of Defense, the U. S. Public Health Service, and other Federal agencies concerned.

Estimate of Needs

1.2 The potential transfusion requirements, based on a large scale enemy attack on this country, are tremendous. They can be met only by proper preparation. The details of casualty estimates for typical American cities, based on an atomic bomb attack, have been presented in *Health Services and Special Weapons Defense*.[a] Estimates for large scale bombing with more conventional weapons would show little difference.

1.3 As a general guide for planning, an average of one unit (500 cc.) of blood, plasma or plasma expanders per casualty surviving after 24 hours, would be needed each week for the first 3 weeks. This

[a] *Health Services and Special Weapons Defense*, 1950. Federal Civil Defense Administration AG-11-1, pars. 1.31 to 1.59, pp. 13 to 21.

is based partly on careful study of the military experience with battle casualties during World War II and partly on the data from Japanese casualties at Hiroshima and Nagasaki. Thus, for every 1,000 casualties, 3,000 units of blood, plasma, or plasma expanders would be required. About one half of this amount (1,500 units) should be whole blood. This is based, to a large extent, on the necessity for using plasma and plasma expanders during the early postattack period when blood is not yet adequately available, rather than on ideal clinical requirements. This takes into consideration the fact that about one third of the casualties will be walking wounded with relatively minor injuries, one third moderately injured requiring short hospital care, and only one third will have a serious degree of injury requiring extensive hospital care. Almost all of the latter group and a portion of the moderately injured group, including those exposed to radiation, will require one or more transfusions. Therefore, the 3,000 units will be given to about 400 to 500 casualties (40–50 percent) surviving after 24 hours. This estimate provides an average of about six units per casualty requiring transfusion. The peak requirements will occur during the first 72 hours and again after the tenth to twelfth day for treatment of radiation sickness.

1.4 In the hypothetical example employed in *Health Services and Special Weapons Defense* a single atomic bomb could, in daylight and with adequate air raid warning and civil defense preparedness, result in 60,000 casualties in a typical American city. Approximately two thirds of these casualties, or 40,000, would survive after 24 hours. This would require an estimated total of 120,000 units—60,000 units of whole blood and 60,000 units of plasma and plasma expanders—for the care of the approximately 13,000 severely injured, and 3,000 to 7,000 of the moderately injured.[4] These figures are used here for purposes of easy reference to other related data in the cited reference. However, recent developments indicate casualties could be considerably higher, possibly as high as 100,000 to 150,000, from a single burst.

1.5 Military experience has shown that, during the first 24 to 72 hours, reliance upon whole blood alone to combat shock in casualties will be impossible. However, whole blood will be required and can be made available before casualties are evacuated to hospitals equipped and staffed to do blood grouping, typing, and crossmatching for group and type specific whole blood transfusions. Therefore, whole blood for use during the first 12 to 24 hours should be proven group O blood.[5]

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[4] *Health Services and Special Weapons Defense* pars. 7.42 to 7.47, pp. 103 to 105.

[5] Proven group O blood is blood in which the usual cell grouping by anti A and anti B serum has been verified by second test using a different reagent (serum) or method.

which can be used without crossmatching (see par 1.17) The basis for estimating this requirement, as well as the local reserves of plasma or plasma expanders needed until outside help can become effective, is discussed in paragraphs 4.5 to 4.13 (See figs 1 and 2, p 11.)

1.6 *Requirements*—Federal civil defense planning for the treatment of mass casualties resulting from enemy attack provides for very large quantities of essential medical supplies as local, State, and Federal reserves For example, mass enemy attacks could successfully strike a number of major cities simultaneously and result in several million casualties surviving after 24 hours On the basis of the blood and plasma requirements discussed in paragraphs 1.3 and 1.4, there should be minimum reserves of 7,500,000 units of plasma and plasma expanders (about 50 percent of each), with administration sets, in existence at one time, and equipment for the collection and administration of 7,500,000 units of whole blood *Local reserves* required by target areas in the United States would amount to one third of this total The basis for estimating these requirements for each target area is discussed in detail in chapter 4 The adequacy of whole blood supply following enemy attack will depend on meeting requirements through mutual aid and mobile support activities

Present Resources

1.7 *Independent Blood Banks*—The present national resources for civil defense blood requirements are primarily potential, rather than real According to the Second Survey of Blood Banks, made by the American Medical Association in late 1950 (AMA Bulletin 83), there were 1,493 hospital blood banks, and 62 nonhospital banks and community centers This means there were some 5,000 general hospitals without well developed blood bank facilities The American Medical Association survey also showed that these independent blood banks have a normal inventory of about 62,000 units of whole blood, and, according to the first survey in 1949, there were only about 200,000 units of plasma in widely scattered areas Listed in the second survey are basic operating data for blood collecting facilities which will provide valuable information for current civil defense planning purposes Present facilities, however are not yet organized to collect large quantities of blood on a coordinated basis in a national emergency and, in many cases, staffs may not be fully aware of the need for emergency expansion

1.8 *Red Cross program*—Present operations of the American National Red Cross include 61 centers, 46 of these are regional blood centers, supplying both civilian and defense blood needs, and 15 are defense centers providing blood for defense purposes only Production capacity for whole blood in the 61 centers in operation November

1952, was about 400,000 bloods per month This collection capacity may be increased by approximately 25 percent on short notice The Red Cross centers are well organized to collect blood on a coordinated basis in a national emergency

1 9 The Red Cross Blood Program was organized to supply blood and plasma to meet normal civilian needs in those areas being served by the program Having been designated by the Office of Defense Mobilization as the coordinating agency for blood collecting for the defense needs of the National Blood Program (See par 1 11), the American Red Cross coordinates blood collection facilities of the program to

(*a*) Supply whole blood and blood for plasma reserves to meet the requirements of the Department of Defense

(*b*) Provide blood for fractionation for the purpose of obtaining immune serum globulin for modification of measles, hepatitis, and poliomyelitis and to provide albumin for the military and civil defense requirements

(*c*) Supply whole blood to meet the needs of civil defense emergencies and to collect whole blood for processing of a Federal reserve stock of plasma for civil defense

1 10 *Military Bleeding Centers*—To assist in the collection of blood required for national defense purposes, the Department of Defense has established blood donor centers in 34 of the larger Army, Navy, and Air Force installations within the United States

1 11 *National Blood Program*—Blood requirements for national defense are being met by the coordinated activity of the Red Cross centers, the military centers, and 30 independent blood banks selected to participate in the program The National Blood Program is further described in paragraphs 2 3 and 3 7

1 12 At present the Nation's whole blood resources are only partially organized for coordinated emergency action Reserves of plasma are also grossly inadequate Creation of adequate reserves will require time and much effort

Civil Defense Program Objectives

1 13 To provide transfusion supplies and equipment for whole blood and reserves of plasma and plasma expanders as indicated in paragraph 1 6, the following three part program will be necessary

(*a*) Procurement of Federal reserves to meet the major portion of these needs since it is neither practical nor economically feasible for each community or State to be self sufficient Minimum reserves should be obtained locally to supply needs in target areas during the first 8 to 10 hours following an attack Reserves of all but whole blood are expected to be delivered in quantity within 4 hours

(b) Assistance by the Federal Government to States and communities in preparation and procurement of limited local reserves of blood plasma and plasma expanders and whole blood transfusion equipment, which will be needed in target areas during the first 8 to 10 hours following an attack

(c) Organization of blood bank and blood donor facilities to collect large amounts of whole blood which will be needed during an emergency

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FEDERAL CIVIL DEFENSE RESERVE PROGRAM

2.1 Federal reserves of plasma, plasma expanders, and equipment for whole blood transfusions must be obtained to meet the requirements of casualties resulting from enemy attack, in accordance with program objectives and requirements (par 1.6) Human serum albumin may be substituted for plasma in future procurement It is the equivalent of plasma for the treatment of shock These will be procured by FCDA through the Joint Armed Services Medical Procurement Agency by agreement with the *Department of Defense* Standards and specifications are described in supplements A and B

Plasma Reserves

2.2 Federal reserves of blood plasma obtained for civil defense will be packaged in the standard Armed Forces dried plasma unit (500 cc size) Blood will be collected by the American National Red Cross in its regional and defense centers and by independent blood banks which have been requested by the Red Cross to assist in this part of the program The blood will be shipped to licensed manufacturers of biological products who are under contract to process plasma for Federal plasma reserves

2.3 The Federal civil defense reserves of plasma and plasma expanders are only one part of the total Federal reserves Civil defense *reserves, together with reserves of the military forces, comprise the* total Federal resources The Office of Defense Mobilization, on recommendation of its Health Resources Advisory Committee, has authority to allocate the total Federal reserves including daily collection of whole blood to meet both *military and civil defense requirements* and to coordinate the entire program This function was established by a directive from the President of the United States to the heads of executive departments and agencies, December 10, 1951, which stated

> "I have asked the Director of the Office of Defense Mobilization to provide within the office a mechanism for the authoritative coordination of an integrated and effective program to meet the Nation's requirements for blood, blood derivatives, and related substances

ORGANIZATION AND OPERATION OF STATE AND LOCAL PROGRAM

3.1 During the pre disaster phase, the State and local civil defense blood program should consist of

(*a*) Organization of existing blood bank and donor center facilities, including development of needed additional facilities, which can become immediately effective in event of an enemy attack, and

(*b*) Procurement of local reserves of blood plasma, plasma expanders, and equipment for collection and transfusion of whole blood to meet urgent needs during at least the first 8 to 10 hours in target areas

3.2 Within 4 hours, Federal reserves of plasma and plasma expanders will be available in substantial quantities in the disaster area and additional supplies of bleeding equipment will be delivered to mutual aid and mobile support areas Within 8 to 10 hours supplies of whole blood will begin to arrive in quantity from mutual aid and mobile support areas For adequate mobilization of transfusion supplies, current records should be maintained of the available supply of blood, plasma plasma expanders, and equipment reserves at both State and local levels

3.3 During the disaster phase, State and local civil defense agencies will assume control of the State and local organizations of blood bank and blood donor facilities to carry out the planned program of coordinated blood procurement Civil defense agencies need not interfere with the normal operation and control of these independent blood banks and Red Cross facilities, except in civil defense emergencies Because Red Cross centers and those blood banks participating in the National blood program have a Federal responsibility to the Armed Forces and FCDA, their utilization by State civil defense agencies in an emergency must always be cleared through FCDA Mutual aid and mobile support plans for the blood program should follow a pattern similar to that developed for other medical supplies and personnel

Federal Staff

3.4 The health services division of FCDA is in charge of the program This staff maintains liaison with the Office of Defense Mobili

"At his direction the Health Resources Advisory Committee, Office of Defense Mobilization, has established a Subcommittee on Blood for this purpose. This subcommittee will be concerned with the development of a single National Blood Program encompassing all phases of the problem.

"I desire that other departments and agencies of the Federal Government coordinate their activities in the blood field through this mechanism."

Plasma Expanders

2.4 An adequate level of reserve supplies for emergency treatment of shock, in event of mass casualties, can be attained more rapidly only if all acceptable plasma expanders are procured as processing facilities become available. At present, specifications have been approved for *dextran* and *PVP* (polyvinyl pyrrolidone), which although not entirely equal to plasma, are quite satisfactory for emergency use under disaster conditions. Production facilities have now been developed, and appreciable amounts are available in Federal civil defense reserves. Both of these expanders have been recommended by the National Research Council: dextran for general emergency use and PVP for war emergency use only. The former has also been cleared by Food and Drug Administration for interstate commerce. Other satisfactory plasma expanders may become available at a later date. (See also par. 5.4.)

Equipment for Whole Blood Transfusion

2.5 Federal reserves of equipment required for mass whole blood transfusions must be procured to meet civil defense requirements. These reserves will include expendable blood donor sets, recipient sets, bleeding bottles, vacuum type, containing anticoagulant preservative solution), sets of anti A, and anti B, and anti Rh (D) typing sera, and insulated cases for shipment of refrigerated blood. High titered group O serum will also be procured for proving group O bloods, as well as 30 percent bovine albumin for the combined ABO and Rh crossmatch technique.

Storage of Reserve Supplies

2.6 Strategically safe storage of Federal civil defense reserves will be provided in accordance with a carefully planned program, which will include rapid transportation facilities to target areas and blood procurement centers. The typing sera will be obtained in the dried state. In addition, to avoid loss due to outdating, plans must be made for continuous use and replacement of reserve supplies through existing blood programs.

THE STATE AND LOCAL WHOLE BLOOD PROGRAM

4 1 The importance of careful plans and preparations which will result in effective utilization of all available blood bank and blood donor facilities must be realized This is essential if an adequate amount of whole blood is to be provided for the treatment of the large number of serious casualties that could result from enemy attack

Existing Facilities

4 2 The first step is to provide for emergency expansion of the larger, well organized blood bank groups to the maximum extent. This includes State, community, or regional blood bank services, whether a part of the Red Cross Blood Program or an independent organization. The next step is to bring into the organized disaster plan the larger individual hospital blood banks, and expand them to the maximum extent.

Additional Facilities

4 3 Finally, on the basis of a survey of potential facilities and requirements by the State committees, the smaller blood banks should be developed and expanded further and made a part of the program In addition, emergency blood donor facilities should be prepared to take advantage of every population group that could be made available For practical purposes, consideration should be given to population groups that would provide potential blood donors of 500 or more Many of these groups can be accommodated most easily by emergency mobile units from blood banks or donor centers in nearby large communities

Training of Personnel

4 4 To man these expanded blood bank and blood donor facilities on a 24 hour basis, it will be necessary to recruit and train periodically physicians as directors of centers or banks, nurses, nurse's aides, technicians, and orderlies In many cases, students in these or related fields can be used Personnel requirements will vary from

zation and works closely with all Federal agencies and national organizations concerned These include the Department of Defense, *National Institutes of Health of the U S Public Health Service*, American National Red Cross, American Medical Association, Association of State and Territorial Health Officers, American Hospital Association, American Society of Clinical Pathologists, and American Association of Blood Banks.

State Staff

3 5 *On the recommendation of the State's civil defense director of* health services, each State civil defense director should appoint a blood program director and an advisory committee, to assist in developing the civil defense blood program The committee should include representatives for participating organizations including the State Medical Association, the American National Red Cross, the State Hospital Association, the State Health Department (*if not already represented by a full time staff member assigned to civil de*fense), and the American Association of Blood Banks This committee should take an active part in the pre disaster phases and should be prepared to assist the director in event of an enemy attack

Local Staff

3 6 Each local civil defense director should appoint a blood program director and an advisory committee This committee should have representation similar to that of the State staff except that county or local units of the organizations listed in paragraph 3 5 (County Medical Society, City or County Health Department, Red Cross Chapter, etc.), should be represented

National Blood Program

3 7 FCDA, through the National blood *program*, is *actively* engaged in a long term, broad gage public education program in conjunction with the Office of Defense Mobilization, Department of Defense, and American National Red Cross, with the cooperation of the Advertising Council All media, including the use of the new mass media, such as closed circuit television, and all national organizations are involved.

3 8 State and local civil defense directors should avail themselves of existing and planned material, and wherever possible, enlist local support for tie in with national advertising coverage from the Advertising Council

(*c*) Thus when the basic estimates are corrected to allow for treatment of casualties who do not survive the first 24 hours the figures per 1,000 casualties in (*a*) become 3,125 units (1,525 blood and 1,600 plasma or plasma expanders

4 6 In the event of mass casualties civil defense medical and rescue units can expect to have evacuated at the end of 8 to 10 hours, approximately 40 to 50 percent of the casualties to first aid stations and improvised hospitals This will include the great majority of the walking wounded—that third of the 1 000 casualties (300 to 350) who have sustained only relatively minor injuries It is also estimated that 80 to 90 percent of the casualties will have been evacuated within 18 to 24 hours During this period the majority will be in the seriously injured group, thus the first 72 hours will be the period of maximum transfusion requirement

4 7 Therefore, of 1 000 casualties surviving after 24 hours approximately 400 to 500 would be evacuated to first aid stations and improvised hospitals during the first 8 to 10 hours plus about 50 of the fatally injured group Approximately 35 to 40 percent (190 to 250) will be more or less seriously injured and can be expected to require an average of two transfusions each during this period Thus total requirements per 1,000 casualties surviving after 24 hours for local reserves would be approximately 425 to 525 units of blood, plasma, and plasma expanders Since little whole blood will be available in this early period, at least 85 percent of this amount (350 to 440 units) should be local reserves of plasma or plasma expanders The remaining 15 percent (70 to 80 units) should be proven group O blood which can be supplied by nearby mutual aid activities Transmission supplies would be supplemented from Federal reserves and, before the end of 8 to 10 hours, mutual aid and mobile support activity would enable subsequent requirements for whole blood to be met

4 8 Minimum requirements for the remainder of the first 72 hour peak demand period should be based on the estimate that there will be an additional 200 to 300 moderately or seriously injured casualties per 1,000, who will require an average of 3 transfusions, making a total number of about 400 to 550 per 1,000 casualties who will require 1 or more transfusions Therefore, a minimum of 900 units should be provided for use during the remainder of the first 72 hour period (10 to 72 hours), or a combined total of 1,250 units for the 72 hour period (fig 1) Most of the 900 units (60 to 70 percent would be desirable) should be whole blood and facilities should be made available for use of group and type specific blood in hospitals by the end of the first 24 hour period

4 9 Since adequate reserves of plasma and plasma expanders are not now available, plans must be made to meet the transfusion needs

additional workers for large established facilities to stand by staffs for emergency blood donor facilities in smaller towns Number and type of persons required and factors which should be considered in their training are discussed in the cited references[6,7] All auxiliary personnel should receive a general course of instruction in practical work and then be trained to perform a specific function in emergency blood collecting Training should be given in established blood banks and Red Cross centers Periodic refresher training must be carried out if proficiency is to be maintained Specialized training for the blood program should be supplemented by the general basic training for all civil defense workers, either before, during, or after the specialized training

Estimate of Immediate Whole Blood and Plasma Requirements

4 5 For estimating minimum requirements in target areas the following data may be used

(*a*) *3,000* units (*1,500* of blood and 1,500 of plasma and plasma expanders) would be required for the first 3 weeks per 1,000 casualties surviving after the first day

(*b*) 40 to 50 percent of those surviving after 24 hours (400 to 500 casualties) would require one or more transfusions (an average of six units per casualty)

(*c*) There would be one or two peak demand periods—first, the needs of the seriously injured during the first 72 hours, and second, the treatment of radiation illness after the tenth or twelfth day if there are appreciable numbers of such casualties The immediate needs during the first 8 to 10 hours, which must be met by locally available supplies, can thus be calculated with reasonable accuracy

(*d*) *These basic estimates have been intentionally based on casualties* surviving after 24 hours, ignoring the one third who do not survive this period An estimated 10 percent of this fatally injured group (50 in addition to each 1,000 casualties surviving after 24 hours) will be brought into first aid stations alive and treated Minimum requirements can be adjusted for this group of casualties by increasing amounts of whole blood and plasma (or plasma expanders) required during the first 8 to 10 hours by about 0 5 units of blood and 2 units of plasma for each of the 50 added casualties—or 25 units of blood and 100 units of plasma This would increase the 8 to 10 hour requirements per *1,000* casualties surviving after 24 hours (par 4 7) to 425–525 units, of which about 70 to 85 should be whole blood, and 350 to 440 plasma, or plasma expanders

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[6] *Blood Transfusion* chs 24 and 25 pp 46_ to 514

[7] *Blood and Plasma Transfusion* ch 8 pp 300 to 349

type specific transfusion within 24 hours, if possible Plans must be made in each target area to meet the urgent 8 to 10 hour requirements through mutual aid agreements with nearby communities The total requirements, including the 72 hour peak demand, will have to be met through mutual aid and mobile support This will often involve regional offices for interstate support, and at times the Federal office for interregional support.

Amounts of Storage of Local Reserve Equipment

4 11 The amounts of local reserve equipment for the collection and administration of whole blood should be estimated for each target area, using as a guide the factors discussed in paragraphs 4 6 to 4 9 From these data, one should plan for a minimum of 45 to 60 whole blood transfusion units per 1,000 casualties (who survive after 24 hours) for immediate use and for at least 70 to 80 per 1,000 total casualties Before the end of the first 8 to 10 hour period, supplies of equipment will have become available from Federal reserves and shipments of whole blood will have begun to arrive from mutual aid and mobile reserve areas. The local whole blood equipment reserves must, however be stored within the target center and mutual aid area where existing blood banks or emergency donor centers will collect the required blood

4 12 Plans should be made for continuous use and replacement of local reserves of the dried blood grouping and typing sera (par 2 5) through local blood bank programs to avoid loss due to outdating These sera will, of course, need to be used first in the donor centers and later in the hospitals as group and type specific transfusions become possible Other items in the whole blood transfusion units (bottles, administration and donor sets) do not have a dating period Their shelf life is long, at least 10 years, and therefore rotation for use and replacement to minimize loss from deterioration, should be undertaken only when economy will result. That is, the cost of the rotation over a period of several years should be less than the cost of periodic discard and replacement

4 13 Reserve equipment required for collecting and administering whole blood must conform to standard specifications or be interchangeable with standard equipment to be effectively used in a national defense program Minimum requirements of the National Institutes of Health and military specifications have been adopted for this purpose (supps A and B)

The Collection and Processing of Whole Blood

4 14 The standard procedures and techniques described in supplement C have been adopted for American Red Cross blood donor centers, the Armed Forces, and civil defense These have been care

almost entirely through whole blood This situation will gradually change as reserves of plasma and plasma expanders become available *

TRANSFUSION REQUIREMENTS
Per 1 000 casualties surviving after 24 hours [1]

	Minimum for 8 to 10 hours		Minimum for 10 to 72 hours		Total for 72 hours		Total for 3 weeks	
	Total units	Units per casualty	Total units	Units per casualty	Total units	Units per casualty	Total units	Units per casualty
Blood [2]	75	0 07	600	0 6	675	0 67	1 525	1 45
Plasma and plasma expanders	400	0 38	300	0 3	700	0 68	1 600	1 52
Total	475	0 45	900	0 9	1 375	1 35	3 125	2 97

OR 1 500 total casualties An allowance has been made for the treatment of those casualties whose injuries prove fatal within 24 hours See par 4 3 (d)

[2] If for any reason whole blood requirements cannot be met appropriate amounts of plasma or plasma expanders will need to be made available

FIGURE 1

AVERAGE NUMBER OF 500 CC UNITS PROVIDED
Per casualty requiring one or more transfusions [1]

	For [illegible] casualties during first 8 to 10 hours (units)	For 300 casualties during first 10 to 72 hours (units)	For 550 casualties during first 72 hours (units)	For 550 casualties total for 3 weeks (units)
Blood	0 34	2 0	1 2	2 77
Plasma and plasma expanders	1 82	1 0	1 3	2 90
Total	2 16	3 0	2 5	5 67

[1] This number is 40 to 50 percent of all casualties or 400 to 500 per 1 000 casualties surviving after 24 hours plus 50 fatally injured

FIGURE 2

Total Whole Blood Requirements

4 10 Using the hypothetical example resulting in 40,000 casualties surviving 24 hours, the total 3 week requirement of whole blood is 61,000 units with an immediate 8 to 10 hour minimum need of about 3,000 units (see par 5 2 for amount of plasma and plasma expanders) Although it will be necessary to use *group O blood almost* entirely during the first day and to a decreasing extent thereafter, every effort should be made to establish facilities for group and

* *Health Services and Special Weapons Defense* pars 1 31 to 1 59 pp 15 to 21 and pars 7 40 to 7 6 pp 105 to 109

(c) The group is proven by an additional test

(d) The blood donation should also be typed with Rh_0 serum if this has not been done

(e) A standard serologic test for syphilis should be performed However this may not always be possible during the first 24 to 48 hours because time, personnel, and facilities may not be available When whole blood transfusions are required as a life saving measure, blood must be employed regardless of whether or not such testing has been done The risk of transmission of syphilis is very small since the spirochete will not survive long in refrigerated blood (less than 72 hours), and effective antibiotic therapy is available

(f) Since about one fourth of group O bloods have high serum titers of A and B agglutinins (1 to 200 or more by the test tube method), they cannot be used with entire safety for all recipients of other groups Therefore, the serum from each proven group O blood collection should preferably be titered against known A and B cells (according to the technique described in *Blood and Plasma Transfusions*), and high titered bloods so marked, if time, availability of technicians and facilities permit It is anticipated that there may not be personnel and facilities sufficient to carry out this procedure during the first 24 to 48 hours after an attack

(g) Group O blood collections should be labelled Proven Group O, or High titered Proven Group O, for Group O Recipients Only, if found to be high titered after performing the testing as outlined in step (f), Rh Pos (or Neg), and the results of the serologic test for syphilis indicated appropriately (See supp C, figs 2 and 4, pp 148 and 149)

(h) High titered group O blood (or all group O blood) may be treated with A and B specific substances, if desired and if they are available, for increased safety in universal use without crossmatching However, reserve supplies of these substances are limited by the relatively short dating period of the presently available material

4 19 From a practical point of view, it may be necessary to administer proven group O bloods to all recipients without ABO crossmatching and without regard to Rh compatibility, at least during the first 12 to 24 hours However this is not an entirely safe procedure with all recipients and is not desirable medically if it can possibly be avoided Therefore, the following suggestions are given with the recommendation that they be carried out to the greatest possible extent,

(a) If titrations can be done, high titered group O blood should be given to known group O recipients

(b) Young women should be given Rh compatible or Rh negative blood, if practicable, to avoid the risk of possible sensitization to the

fully worked out through joint consultation of the National Institutes of Health of the U S Public Health Service, the American National Red Cross, the Department of Defense, and FCDA These procedures and techniques for the collection and processing of blood were established on the basis of actual experience since the start of the Korean conflict in June 1950 The instructions in supplement C have been adapted for use in civil defense emergencies and should be followed in detail Employment of such uniform standards is considered essential for the widespread coordinated collection and use of the large amounts of whole blood which will be necessary However, it is recognized that strict adherence to these standard procedures may not be possible in large scale disasters, particularly in the immediate disaster area Specific recommendations regarding practical, compromise, emergency procedures will be published as a supplement to this manual

4 15 Because it may not be possible to follow the standard procedures in supplement C during and immediately following a civil defense emergency in or near the disaster area, the Committee on Blood and Related Problems of the National Research Council has prepared for civil defense use supplement D, "Emergency Blood Transfusion" This represents the minimum in standards and procedures which can be considered acceptable in acute emergency situations

4 16 Supplement E, the Red Cross Central Supply Room Manual, describes standard techniques in the preparation of donor center sterile supplies It should be of particular value for training auxiliary personnel to serve in this area during an emergency

Emergency Use of Group O Blood

4 17 Plans must be made to use proven group O blood for whole blood transfusion in a civil defense emergency for the first 12 to 24 hours as is done in our military services under similar circumstances The reason for this is that it will be extremely difficult, if not impossible, to provide immediate laboratory facilities for blood grouping, typing, and crossmatching in emergency hospitals An excellent and useful discussion of the use of group O blood, applicable to disaster conditions, is found in supplement D and *Blood and Plasma Transfusions*[9]

4 18 Processing of group O blood for emergency administration without crossmatching is carried out as follows (see supp D for details)

(*a*) Selection of donors and collection of their blood

(*b*) The group of the blood donation is then rechecked by testing the cells with anti A and anti B serum

[9] *Blood and Plasma Transfusions* ch 10 pp 3 0 to 363

be carried out among the potential blood donors in selected areas near target areas The purpose of this program would be to identify in advance a reasonable number of voluntary group O donors in locations where they are likely to be available for emergency bleeding at hospitals or stand by donor centers designated to act in immediate support of the target areas Identification cards or tags should be issued and a roster of these emergency donors maintained (preferably in dispersed points) and kept up to date by periodic revision This procedure would simplify the provision of an initial supply of group O blood by reducing up to 50 percent the number of donors who would have to be processed to meet the emergency quota before outside help became available All existing blood banks and donor centers can assist in this program in the course of their normal operations by issuing identification cards to donors, especially group O donors, and urging them to volunteer for the local civil defense blood donor panel Proving of all group O blood for probable use without crossmatching must be done at the time of actual donation Anti A, anti B, and anti Rh_0 sera should be employed so that Rh negative blood can be used where possible, as discussed in paragraph 4 18 Such a program could well be made a part of the preparation of local emergency reserves of blood plasma in target areas

Mass Grouping Not Recommended

4 24 A national program of mass grouping of the population is not recommended, although the limited blood grouping program described above is advised as a sound civil defense procedure There are valid reasons for this

(*a*) There is no certainty that the blood group identification would be available when needed, even tattoo marks can be destroyed by burns or other injury

(*b*) An attempt to employ group specific blood during the first 24 hours following an enemy attack would greatly complicate the supply problem in servicing large numbers of first aid stations and emergency hospitals and would require, at the very least, facilities for cross matching wherever blood was to be used

(*c*) Realistic appraisal of the problem of transfusing mass casualties indicates that reliance must be placed on proven group O blood to be used without crossmatching, as is done successfully by our military services under like circumstances

(*d*) It would be a relatively expensive procedure to carry out a careful and accurate mass grouping program

(*e*) Mass grouping of the whole population has in fact a very low priority in community preparation in comparison to adequate sup

Rh factor This sensitization is important since it may interfere with subsequent childbearing

(c) Women who have had children should also receive Rh compatible or Rh negative blood since they may have become sensitized to the Rh factor and could therefore develop a hemolytic crisis with a single Rh incompatible transfusion

(d) All those who have received multiple blood transfusions should also receive Rh compatible or Rh negative blood, if possible, as they too may have been sensitized to the Rh factor

(e) There is no need for concern in transfusing men who have not had multiple transfusions or women past the childbearing age who have not had children or multiple blood transfusions, as it takes at least 1 or 2 weeks for Rh sensitivity to develop in susceptible persons

4 20 These recommendations, which should be carried out, if possible, must not be construed to mean that whole blood transfusion should be denied if it is truly a life saving measure, even though Rh negative blood is not available or Rh compatibility cannot be determined However, in such instances, one should be quite certain that the use of additional quantities of plasma or plasma expanders would not be sufficient to maintain life until compatible blood can be provided

4 21 However, exclusive use of large amounts of proven O Rh negative blood in the types of cases outlined here is utterly impossible, and further, exclusive use of group O bloods for large numbers of casualties over much more than a 24 hour period can result in a serious shortage of blood for group O patients Therefore, every effort must be made to establish facilities for use of group and type specific blood at the earliest possible time, preferably within 24 hours

4 22 In storing and shipping blood under these emergency conditions, every effort must be made to cool the blood to 4° to 10° C (preferably 4° to 6° C) as rapidly as possible and to maintain this temperature until the blood is administered If standard insulated blood shipping containers and refrigerators for storage are not available, other substitute methods can be used, such as the employment of any container that will hold cracked ice[10] The freshest blood available should always be shipped for emergency use Supplement C contains detailed shipping instructions

Advance Grouping of Potential Blood Donors

4.23 Consideration should be given to a selective blood grouping program to provide a ready supply of group O blood to meet the needs for the first 8 to 10 hours in target areas This program should

[10] *Blood Transfusion* ch 14 pp 341-346

be carried out among the potential blood donors in selected areas near target areas The purpose of this program would be to identify in advance a reasonable number of voluntary group O donors in locations where they are likely to be available for emergency bleeding at hospitals or stand by donor centers designated to act in immediate support of the target areas Identification cards or tags should be issued and a roster of these emergency donors maintained (preferably in dispersed points) and kept up to date by periodic revision This procedure would simplify the provision of an initial supply of group O blood by reducing up to 50 percent the number of donors who would have to be processed to meet the emergency quota before outside help became available All existing blood banks and donor centers can assist in this program in the course of their normal operations by issuing identification cards to donors, especially group O donors, and urging them to volunteer for the local civil defense blood donor panel Proving of all group O blood for probable use without crossmatching must be done at the time of actual donation Anti A, anti B, and anti Rh_o sera should be employed so that Rh negative blood can be used where possible, as discussed in paragraph 4 18 Such a program could well be made a part of the preparation of local emergency reserves of blood plasma in target areas

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plies of such items as whole blood transfusion units, plasma and plasma expanders, burn dressings, and other first aid stations supplies Therefore, reserve supplies for the treatment of casualties should have priority in the expenditure of funds Mass grouping programs, in spite of their popular appeal, can logically be undertaken only if surplus funds remain.

THE STATE AND LOCAL PLASMA AND PLASMA EXPANDERS RESERVE PROGRAM

5 1 A supply of locally produced liquid, frozen, or dried plasma (including the approved plasma expanders, dextran and PVP) should be developed in each target area for the immediate care of casualties This supply should be sufficient to enable each hospital (existing or improvised) to provide a minimum reserve based on a realistic analysis of potential casualties in terms of the probable rate of rescue and evacuation during the first 8 to 10 hours Additional supplies will have become available from Federal reserves and from mobile support activities before the end of this period This local reserve should be made available for immediate use both in hospitals and first-aid stations

Estimate of Amounts of Plasma Required

5 2 As discussed in paragraphs 1 3 and 4 5, the requirements for plasma and plasma expanders for the treatment of casualties during the first 3 weeks following an enemy attack are 1,500 units per 1,000 casualties surviving after 24 hours (1 5 units per casualty) or 1,600 units per 1,500 total casualties The minimum requirements during the first 8 to 10 hours (fig 1, p 11) are estimated to be 350 to 440 units (about 0 38 unit per casualty), adjusting to include allowances for the casualties not surviving after 24 hours (par 4 5) This is the amount that should be immediately available locally, including mutual aid potentials

5 3 Translating this to the hypothetical example of 40,000 casualties surviving after 24 hours, the total 3 week requirement would be 64,000, with an immediate 8 to 10 hour minimum need of 16,000 units Plans should be made in each target area to obtain through local donor recruitment and processing, or through purchase, the urgent 8 to 10 hour requirements of plasma and plasma expanders, including in the plans the full utilization of immediate mutual aid facilities It is anticipated that Federal reserves of plasma and plasma expanders will be so located that significant amounts can be made available in target areas within 4 hours following an attack

Plasma or Plasma Expanders

5 4 Blood plasma is, of course, preferable to any of the known plasma expanders, since the latter are foreign substances whose function is limited to expanding the blood volume temporarily (6 to 12 hours) However, since this is the critical problem in the treatment of shock and the acceptable plasma expanders are clinically effective, they are in themselves adequate therapeutically except in the patient with severe loss of blood (or of plasma in severe burns) In this type of casualty, whole blood or plasma is usually required to maintain life after the first 10 to 12 hours, even though the blood volume has been restored for the time being by a plasma expander

5 5 *Dextran* (of the type approved by the National Research Council and cleared by the Food and Drug Administration) is the only completely acceptable substitute for emergency field use at present

5 6 PVP (polyvinyl pyrrolidone) has been given limited approval by the National Research Council and recommended for reserves for war emergency use It has not been cleared by the Food and Drug Administration for interstate commerce

5 7 Both dextran and PVP are included in the Federal reserve stocks and both are eligible for State procurement under the Federal contributions program

5 8 *Ossein gelatin* (of the approved and licensed Knox P 20 type) is also acceptable clinically, but the fact that it is jelled at normal room temperature and, therefore, requires heating before and during administration, makes it generally unsuitable for use in disaster conditions Additional plasma expanders are currently under investigation and consideration for approval

5 9 A statement prepared by the Subcommittee on Shock of the National Research Council (supp F) outlining the role of *saline* in the treatment of shock, should be utilized for guidance in the parenteral and oral use of this agent

Local Preparation of Plasma

5 10 The local preparation of plasma reserves should be undertaken in all target areas Amounts should be based on the estimates described in paragraph 5 2 State and local programs should be developed and carried out under direction of State and local blood program directors of civil defense health services The techniques of preparation must conform to the Minimum Requirements of the National Institutes of Health (supp A) Acceptable methods of preparation are described in selected standard reference textbooks [11, 12]

[11] *Blood Transfusion*
[12] *Blood and Plasma Transfusions*

5 11 The civil defense blood program director must coordinate the recruitment of donors with the American National Red Cross and community blood programs Conflicting donor recruitment programs within the same geographic areas should be properly coordinated into a joint recruitment program meeting all needs In those areas where the Red Cross is recruiting donors for the Federal defense requirements, arrangements should be made with the Red Cross to increase quotas to provide blood for local reserves of plasma, since the Federal requirements must be met

Techniques To Be Employed

5 12 The preparation and storage of dried, frozen, and liquid plasma by methods suitable for local processing of plasma reserves are adequately described in detail in the references cited [13, 14]

5 13 *Dried plasma* is the preparation of choice if proper facilities exist locally or can be contracted for in a licensed laboratory In the latter instance, either whole blood or bulk (pooled) liquid plasma may be shipped for processing, depending on the particular circumstances Limited drying facilities are available in several parts of the country, and, on request, FCDA will assist in making such arrangements for target areas wherever possible The principal advantages of the dried product are its stability, ease of storage and transportation, freedom from danger of contamination, and its long dating period (5 years)

5 14 *Frozen plasma* is probably second choice from an ideal point of view and is recommended over liquid plasma where large amounts are to be stored over a long period of time It has the disadvantage, compared to the dried form, of requiring −20° C storage facilities and adequate 37° C water bath equipment for the necessary rapid thawing before administration Thawing can be done at the storage point before shipment for immediate use, or at the point of use if the plasma is transported and maintained at −20° C The dating period is also 5 years

5 15 *Liquid plasma*, the form commonly employed in most hospital blood banks, is satisfactory if meticulously prepared It is easy to store in relatively small amounts since only maintenance of normal room temperature is required Also, in contrast to frozen plasma, it may be used immediately without further processing However, liquid plasma is the most hazardous form for storage since it must be *completely sterile* Unlike the dried or frozen forms, a few chance contaminating organisms will grow in this ideal culture medium and make the plasma unfit for use While loss from contamination is

[13] *Blood Transfusion* chs 15 16 17 and 18 pp 347 through 419
[14] *Blood and Plasma Transfusions* chs 7 and 8 pp 213 through 298

minimal when liquid plasma is prepared by experienced personnel with proper equipment and techniques, this hazard must not be regarded lightly In the preparation of liquid plasma for storage at room temperature, 50 percent dextrose always should be added to make a final concentration of 5 percent to minimize flocculation[14] The dating period of liquid plasma is 2 years

5 16 At present, the Minimum Requirements of the National Institutes of Health (supp A) require that all plasma be treated by ultraviolet irradiation This should be done in the preparation of local civil defense reserves whenever suitable arrangements can be made with a laboratory having proper facilities Since these laboratories are limited, arrangements for this treatment will not always be possible FCDA will assist in arranging for processing in the same manner as indicated for preparation of dried plasma

Standard Equipment

5 17 For disaster purposes equipment used locally for the final container of plasma and for administration sets must conform to or be interchangeable with that employed for whole blood, as described in supplement B, standard specifications for the national defense blood program Only in this way can FCDA make certain that plasma reserves from any community will be administered with ease and dispatch in all parts of the country

Storage of Plasma

5 18 The majority of the local plasma reserves should be stored in the periphery of target areas, or in relatively safe locations, along with other medical supplies for emergency hospitals and first aid stations Operational supplies for immediate use must, of course, be stored in permanent or fixed emergency facilities Arrangements should be made with local blood banks for rotation by continuous use and replacement of these plasma reserves to avoid loss because of outdating

[14] *Blood Transfusion* p 373

AID TO THE STATES

6 1 Consultation and assistance in the blood program will be available to States and communities from FCDA through its regional offices

Federal Contributions Program

6 2 Federal contributions will be made available to States according to limits established by appropriations for this purpose and by the regulations established by FCDA If the State program includes donor recruitment for plasma reserves and this includes localities where the American National Red Cross is presently recruiting blood donors for the Federal reserves of plasma, State plans must be jointly approved by the American National Red Cross and FCDA to insure proper coordination in the conduct of an expanded joint donor recruitment program to the end that Federal requirements continue to be met. In addition, the Red Cross is prepared to assist actively in donor recruitment by working with local civil defense organizations in other parts of the country

SELECTED MINIMUM REQUIREMENTS[1]

Federal Security Agency
Public Health Service
National Institutes of Health,
Bethesda 14 Md
2d revision August 1 1951

I. Minimum Requirements Citrated Whole Blood (Human)

1 THE PRODUCT

1 1 *Proper name*—The proper name of this product is Citrated Whole Blood (Human)

1 2 *Source*—Only those persons may serve as a source for citrated whole blood whose temperature and blood pressure are normal and who are free of disease transmissible by blood transfusion, particularly malaria, syphilis, and acute upper respiratory diseases, as far as can be determined from the donor's history and from such physical examination and clinical tests as appear necessary for each donor on the day the blood is obtained As an added precaution a history of viral hepatitis shall be cause for rejecting the donor Existing pregnancy or pregnancy within the preceding 6 months shall disqualify a donor

1.21 Only persons having not less than 12 5 gm of hemoglobin per 100 ml of blood shall serve as donors If the copper sulfate specific gravity method is used to determine hemoglobin, a specific gravity of not less than 1 053 shall be used as indicating an adequate hemoglobin level[2]

2 COLLECTION OF THE BLOOD

2 1 *The bleeding clinic*—Determination of the suitability of the donor and drawing blood from the donor shall be the responsibility of a licensed physician and shall be done by him or under his supervision[3]

[1] Selected Minimum Requirements (I through V) reprinted from Minimum Requirements Laboratory of Biologics Control National Institutes of Health Bethesda Md

[2] *Measurements of Specific Gravity of Whole Blood and Plasma by Copper Sulfate Solutions* Robert A Phillips Donald D Van Slyke Paul V Hamilton Vincent P Dole Kendall Emerson Jr and Reginald M Archibald. J Biol. Chem 183 No 1 305–360 1950

[3] Amendment of August 20 1952.

with the maintenance of the general sanitary standards required. The drawing may be performed in a suitable bleeding room in the laboratory or in a blood bleeding station or some other place having suitable space at bleeding point. Irrespective of the place of bleeding, the personnel engaged in drawing blood, the space and the equipment involved remain the responsibility of the processing laboratory under whose license the blood is to be processed.

2.2 *The anticoagulant solution.*—The apparatus used for drawing the blood and the receiving unit shall be chemically clean, pyrogen free and sterile. The receiving unit shall contain a pyrogen-free anticoagulant solution of the following composition:

	Solution A	Solution B
Trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	22.0 gm.	13.2 gm.
Citric acid ($C_6H_8O_7 \cdot H_2O$)	8.0 gm.	4.8 gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	24.5 gm.	14.7 gm.
Water for injection (U.S.P.) to make	1,000 ml.	1,000 ml.

For each 100 ml. of blood to be drawn add 15 ml. of solution A or 25 ml. of solution B to the receiving unit before sterilization. Sterilization shall be by autoclaving in a manner that will not damage the dextrose. The pH of the anticoagulant solution is 5.0. An intermediate volume of anticoagulant solution may be used provided the formula is adjusted so that the above ratios between the components and each 100 ml. of blood drawn are maintained. Approval for the use of another formula will be given upon presentation of evidence that the new formula is as satisfactory as the above.

2.21 Bleeding bottles and donor sets used for the collection of citrated whole blood (human) shall be identified by lot number and shall have been shown to be pyrogen free and sterile.[4]

2.3 *Donor identification.*—A system which carries through from donor to recipient must be used to identify the blood. This can best be accomplished by a suitable label or tie tag filled out for the donor at the time of completing the donor's history card. The label and history card should carry the same identification or lot number. This label or tie tag accompanies the donor to the bleeding room and is applied to the bleeding bottle while it is still possible to check accurately the identity of the blood with the donor and his history card.

2.4 *Protection of donor.*—The preparation of areas of the skin used for the collection of blood or for injections incidental to blood collection shall be adequate to protect the donor against infection. Apparatus or instruments such as lancets, needles, syringes, or other blood letting devices capable of transmitting infection to the donor shall be heat sterilized prior to use for each donor. Heat sterilization shall be by autoclaving for 30 minutes at 121.5° C. (15 lb. pressure), by dry heat for 2 hours at 170° C., or by boiling in water for 30 minutes at 100° C. (See Memorandum of Details, 3d rev., June 15, 1945, sec. 2.)

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[4] Amendment of March 13, 1952.

2 5 *Method of bleeding the donor*—The method employed for the removal of blood from the donor must conform to accepted standards of asepsis *The bleeding shall be made into a sterile system, either closed or vented, which must permit the transfer of material entirely within the system without risk of contamination* This transfer is accomplished either by the introduction or removal of sterile air by appropriate changes in pressure at the time of use or by the provision of adequate vacuum in the receiving unit prior to the time of use The bleeding bottle shall have been cooled at 4° to 10° C, when possible, prior to receiving the blood Each bleeding shall be drawn into its own receiving bottle which shall be the same container used later as the dispensing bottle for the whole blood During the bleeding the anticoagulant and the entering blood should be quickly and thoroughly mixed by a swirling motion Violent shaking must be avoided The outside of the bottle of blood must be kept clean and free of blood to protect workers against exposure to disease transmissible by blood [5]

2 6 *Storage*—Immediately after bleeding the blood shall be placed in storage at 4° to 10° C, preferably 4° to 6° C (freezing must be avoided at all times) If transportation of the blood from the bleeding clinic to processing laboratory is necessary, it shall be transported in shipping cases provided with refrigeration sufficient to hold the blood at 4° to 10° C, preferably 4° to 6° C, if it has already been cooled, or sufficient to bring the temperature to the 4° to 10° C, preferably 4° to 6° C, level while in transit if time permits and it has not been precooled to this level Immediately upon receipt at the processing laboratory it shall be placed in storage at 4° to 6° C

2 7 *Pilot samples for laboratory tests*—One properly identified, *sterile pilot tube shall be attached securely to the bottle before bleeding*, and shall not be detached from the bottle until after the blood has been crossmatched and selected for transfusion to a particular recipient Additional pilot tubes for laboratory testing should also be collected All pilot tubes must be identified in the same manner as the bottle of blood prior to the collection of the blood and the samples shall be collected by the individual collecting the blood

3 Preparing the Blood for Use

3 1 *The serological test*—*An acceptable serological test for syphilis shall be made on a specimen of blood taken from the donor at the time of bleeding*, and the blood shall not be used for transfusion unless the result of the test is negative or the blood has been stored for at least 96 hours as indicated in section 3 11 Interpretation of the serological reaction shall conform to the local legal requirements applicable to serological tests for syphilis or to whole blood transfusions in the State where the test is performed The laboratory performing the test shall be properly qualified or certified if there are local or State agencies to provide such qualification or certification

[5] Amendment of March 1? 195?

3 11 The serological test does not detect the donor whose syphilitic infection is of too short duration to have established a positive serological reaction The donor s history (see 1 2) taken on the day of bleeding may also fail to identify this type of infection Adequate physical examination likewise may be inconclusive and is often impracticable Epidemiological data suggest that the random selection of donors would yield only one bleeding containing viable *S pallidum* in approximately 9,000 bleedings

If the potential donor cannot establish freedom from recently acquired serological negative syphilis to the satisfaction of the physician in charge, it is recommended that the matter be resolved by (*a*) rejecting the donor, (*b*) holding the blood for at least 96 hours, or (*c*) diverting the blood to other uses

3 2 *Determination of the blood group*—Each bottle of citrated whole blood must be classified as to blood group on the basis of tests done on pilot tube samples and the findings must be made a part of the laboratory record and of the final container label Each blood should be tested independently for its group by two workers, or by the same worker using either or both of the two following methods A grouping procedure which minimizes errors and assures accuracy is a single grouping of the donor s red cells against satisfactory grouping serums, followed by a second grouping test which uses the donor s serum against fresh A and B cells A second procedure capable of giving accurate results is to determine the grouping of the donor s cells independently against two satisfactory sets of grouping serums of different lot numbers The result of the tests must be recorded on the bottle label and on the donor s history card This requirement applies to the donors regardless of any previous donation as the record refers to the blood in a particular bottle rather than to a donor A bleeding is not satisfactory for release until duplicate grouping tests are in agreement

3 21 Only anti A and anti B blood grouping serums meeting N I H minimum requirements for these products shall be used and the technique used in grouping shall be that recommended for the serums being used

3 3 *Determination of the Rh type*—Each bottle of citrated whole blood must be typed for the Rh factor, using an anti Rh_0 (anti D) typing serum The result of the test must be recorded on the bottle label and on the donor s history card Subtyping the Rh_0 (D) negative bloods may be carried out if desired, and the finding recorded on both label and history card

3 31 Only anti Rh typing serums meeting N I H minimum requirements for these products shall be used and the technique used in typing shall be that recommended for the serums being used

3 4 *Isoagglutinin titer of group O blood*—The isoagglutinin titers of group O bloods are of importance when group O blood is used for transfusing recipients of other groups Consideration may be given

to reducing these titers by the addition of A and B group specific substances to each bleeding to neutralize the isoagglutinins This practice is not universally approved by immunologists since the injection of these group specific substances may give rise to an increase in the agglutinin titers in individuals of blood groups A, B, or O If group specific substances are added to blood, the amounts added must be stated on the label and in the laboratory records

3 41 An alternate method which avoids the use of group specific substances is the classification of group O bloods as low isoagglutinin titer or high isoagglutinin titer The term "low titer" indicates a blood which has an isoagglutinin titer of not more than 1 200 when determined by the test tube centrifuge method or 1 40 by the well slide method The finding is properly recorded on the donor s record and on the bottle label Under this classification low titer blood may be given to any recipient whereas blood of high titer is reserved for group O recipients or used for purposes other than transfusion

4 General Requirements

4 1 *The sterility test*—Rigid aseptic technique and maintenance *of a sterile closed system from donor to recipient is considered greater* assurance of a sterile product than can be given by sterility tests which necessitate a break in the closed system As an added safeguard, a careful visual inspection of the blood is made in the laboratory just prior to distribution and should be repeated by the clinician before administration On both of these occasions the color and physical appearance must be consistent with freshly drawn citrated normal whole blood

4 11 While a sterility test on each bottle of blood is not required, it is recommended that the technique of handling blood be checked from time to time by performing sterility tests on out dated blood For this test, a sample of not less than 5 ml of well mixed blood is withdrawn aseptically from the bottle and planted in National Institutes of Health thioglycollate broth medium Incubation is at 32° to 35° C for not less than 7 days

4 2 *The final container*—The original bleeding bottle shall also be the final container and shall be made of colorless and fully trans parent *USP type I, II or IV glass The bottle shall be provided* with a closure that will maintain a contamination proof seal until the contents are used Once the closure seal is broken the contents of the bottle should be used promptly or diverted for purposes other than transfusion

4 21 Accessory equipment, including recipient sets supplied by the licensed establishment for the administration of citrated whole blood (human) shall be identified by lot number and shall have been shown to be pyrogen free and sterile[6]

4 3 *The final container label*—*The label shall conform to the labeling requirements of the Regulations* The recommended storage tem

[6] Amendment of March 13 1952

perature should appear in a conspicuous position on the label and in distinctive printing The preferred wording is

"Caution! Keep continuously at 4° to 10° C, preferably 4° to 6° C"

The recommended storage temperature may be expressed in either the Fahrenheit scale or the Centigrade scale (or both) The form of label shown in appendix A is acceptable

4 4 *Directions for using the blood* —The label or an accompanying circular must give adequate directions for administration, call attention to the need for checking the label for proper blood group, the need for careful crossmatching (see Minimum Requirements Anti Rh Typing Serum, Appendix C, sec 1 171), the need for rigidly observing the storage temperature, the need for thoroughly mixing the bottle of blood before performing the transfusion, the absolute necessity for using a filter in the sterile, pyrogen free intravenous administration equipment, the inadvisability of adding any medication to a bottle of blood intended for transfusion, and any other information considered essential The label should also carry the definite statement that the blood should not be warmed before administration

4 41 In the absence of an attached pilot sample for the crossmatching test, it is recommended that the bottle be entered, using aseptic precautions by inserting a sterile hypodermic needle through the closure The required amount of well mixed blood is then withdrawn into a sterile syringe Since this procedure breaks the seal, the bottle of blood should be used promptly

4 42 The filter to be placed in the administration tube must be capable of removing particulate matter of a size potentially dangerous to the patient, but must not cause an undesirable slowing of the rate of blood flow It is recommended that the filter mesh be between 100 and 200

4 5 *Expiration date* —The expiration date for citrated whole blood shall not exceed 21 days from the date of manufacture and this maximum date shall be allowed only if the blood has been stored continuously at 4° to 10° C (preferably 4° to 6° C) The date of manufacture is the date of bleeding the donor

4 6 *Shipment of citrated whole blood* —In order to meet the requirements for safety, purity, and potency as defined by the Regulations, citrated whole blood *must be kept continuously at 4° to 10° C, preferably 4° to 6° C* This is also interpreted to mean while under shipment from the producing laboratory to the user

APPENDIX A

Labels for use with citrated whole blood, packed and resuspended red blood cells

1. Use of Color to Designate Blood Group

1.1 There is no general agreement as to the usefulness of colored labels for whole blood. The decision to adopt colored labels or black and white labels rests with the individual laboratory. There is agreement, however, that if a color scheme is used for this purpose, a uniform system should be adopted by all blood banks.

In order to attain uniformity in blood banks holding U. S. Government license, the following two schemes have been adopted as equally acceptable.

1.2 Labels for use on containers of citrated whole blood and packed and resuspended red blood cells may be printed in black on white paper or colors may be used for blood group designations following either of the two schemes in section 1.3.

1.3 The color scheme may be used in two ways.

(*a*) The entire label is of the color designated for the blood group indicated in section 1.31 with all printing in black ink.

(*b*) Only the blood group designation is printed in the recommended color with all other printing in black ink on a white background. When this scheme is used the blood group designation is printed in distinctive type either in a blocked off section of the label or as under printing through the center of the label. Outline printing in black is necessary in the case of the AB group designation to fulfill the requirement for a white color.

1.31 When a color scheme for differentiating the blood groups is used, the color for each blood group shall be:

		Munsell—Color designation[1]
Blood Group A	Yellow reference color	7Y 8.6/8
Blood Group B	Pink reference color	7RP 4.8/13
Blood Group O	Blue reference color	7PB 3.6/15
Blood Group AB	White reference color	( )

[1] These colors are the same as those in previous minimum requirements; only the system of color designation has been changed.

1.4 (*a*) With the solid color label, section 1.3 (*a*) or the blocked off arrangement, section 1.3 (*b*), the blood group designation should be in bold face type at least one half inch in height.

(*b*) With the under printing design of section 1 3 (*b*) the group designation should be centrally located in bold and distinctly designed type, approximately two thirds the height of the label

1 5 The Rh factor of each blood must be indicated either by a "sticker" label of a size to fit a blocked off space set aside for that purpose on the bottle label or by printing directly on the bottle label in the blocked off space The printing should be black on pure white paper or reverse printing, using bold face type at least one fourth inch high The label for Rh positive bloods should bear the statement "Rh Positive" The label for Rh negative bloods must bear the designation "Rh Negative," and must also indicate the extent of testing by the statement "when tested for Rh, rh′, rh″" appearing only if these tests have been made The C, D, E nomenclature is equally acceptable

1 6 Laboratories operating under U S Government license must comply with the labeling requirements of the Regulations The following design and wording is provided to indicate an acceptable arrangement *Other designs giving the same information may be used* The alternate method of printing the blood group designation is mentioned in sections 1 3 (*b*) and 1 4 (*b*)

Space for Blood Group Designation (see sec 1 3b)	CITRATED WHOLE BLOOD (HUMAN)	Space for Rh Designation (see sec 1 5)

Contains 500 ml human blood plus ______ ml ACD anticoagulant solution. Serologically negative by __________ test Donor No ______ Expiration date ______ ____

CAUTION

Keep continuously at 4 to 10 C preferably 4 to 6 C
Crossmatch before using Administer without warming
A filter must be used in administration equipment
Mix thoroughly immediately before using
Do not add other medication to the bottle of blood prior to administration
Check blood group on label and recipient s group before administration

(Name of laboratory)

U S License No ______ (Address)

For hospital use

Hospital ____________ Patient ____________ Room ________
Recipient blood group ______ Crossmatch by ______ Date ______

[Federal Security Agency
Public Health Service
National Institutes of Health
Bethesda 14 Md
1st revision February 25 1948]

II. Minimum Requirements Packed Red Blood Cells (Human), resuspended Red Blood Cells (Human)

1 Collection of the Blood

1 1 *Selection of the donor*—Only those persons may serve as a source for red blood cells who are certified by a qualified doctor of medicine as being free of disease transmissible by blood transfusion (particularly malaria, other protozoal diseases, syphilis, infectious hepatitis within 6 months, and acute upper respiratory disease) as far as can be determined from the donor s personal history and from such physical examination and clinical tests as appear necessary for each donor on the day upon which the material is obtained from the individual Existing pregnancy or pregnancy within the past 6 months, should disqualify a donor In order to protect the health of the donor and to provide the recipient with a red cell replacement having an acceptable amount of hemoglobin, it is recommended that only those persons may serve as donors who have not less than 12 5 grams of hemoglobin per 100 ml of blood The copper sulfate specific gravity method is approved for this test because of its accuracy and simplicity, a specific gravity of 1,053 or greater indicating adequate hemoglobin The following form shall be used

I certify that the above named donor(s) appear(s) to be free of diseases transmissible by blood transfusion on this date which is also the day of removing the blood from the donor(s)

__________ (Date) __________ (Name of Physician)

1 2 *Method of bleeding the donor*—The method employed for the removal of blood from the donor shall conform to the accepted standards of aseptic surgery and shall be made in a closed system A closed system permits the transfer of material from one container to another entirely within the system without contamination It is accomplished through an exchange of the position of the material within the system, through the introduction of sterile air as the transfer is being made by the application of negative pressure, or by providing the correct amount of vacuum in the receiving unit in advance of its use

1 3 *The anticoagulant solution*—The apparatus used for the removal of the blood and the receiving unit shall be chemically clean

and sterile. The receiving unit shall contain a pyrogen free anticoagulant solution of the following composition, or another formula possessing no less anticoagulant and red cell preserving properties.

	Solution A	*Solution B*
Tri-sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) . .	22.0 gm	13.2 gm
Citric acid ($C_6H_8O_7 \cdot H_2O$)	8.0 gm	4.8 gm
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	24.5 gm	14.7 gm
Water for injection (USP) to make	1,000 ml	1,000 ml

Of solution A add 15 ml, or of solution B add 25 ml, to the receiving unit before its sterilization for each 100 ml of blood to be drawn. The solution can be sterilized by autoclaving without damaging the dextrose. The pH of the anticoagulant solution is 5.0 and becomes adjusted to the optimum when mixed with whole blood. An intermediate volume of anticoagulant solution may be used provided the formula is adjusted so as to maintain the same ratio between the components and each 100 ml of blood drawn.

1.4 *The serological test.*—An acceptable serological test for syphilis shall be made in a qualified laboratory on a specimen of blood taken from the donor at the time of bleeding and the blood shall not be used for the production of citrated whole blood unless the result of the test is negative. Interpretation of the serological reaction should conform to the legal requirements applicable to whole blood transfusions in the State where the blood is to be used. For citrated whole blood licensed under Federal law, the interpretation conforms to that recognized by the National Institutes of Health.

1.41 The serology test does not identify the donor whose infection is of too short duration to have established a positive serological reaction. The donor's history (see 1.1) taken on the day of bleeding also may fail to identify this type of infection. Adequate physical examination is likewise not conclusive and is often impracticable. Epidemiological data suggest that the random selection of donors would yield only one bleeding containing viable *S. pallidum* in approximately 9,000 bleedings.

1.42 No spirocheticidal substance has demonstrated its usefulness for routine use in freshly drawn blood, though well controlled experiments indicate that a trivalent organic arsenical is effective in high dilution. Other studies indicate that *S. pallidum* loses its infectivity within 96 hours in stored blood.

1.43 If the potential donor cannot establish, by history, freedom of recently acquired serologically negative syphilis to the satisfaction of the physician in charge, it is recommended that the matter be resolved by (*a*) rejecting the donor, (*b*) holding the bleeding for at least 96 hours, or (*c*) diverting the blood to plasma where the processing delay will render the spirochete nonviable.

1 5 *The bleeding clinic*—Determination of the suitability of the donor and drawing blood from the donor shall be the responsibility of a licensed physician and shall be done by him or under his supervision[7] with the assistance of the necessary trained attendants The drawing may be performed in a suitable bleeding room located in the licensed laboratory, or some other place having suitable space and equipment Irrespective of the place of bleeding, the personnel engaged in drawing the blood and the space and equipment involved must remain a responsibility of the processing laboratory under whose license the blood is to be processed

2 Preparing the Red Cells for Use

2 1 *Collection and storage of the cells*—Packed red cells and resuspended red cells are byproducts obtained from the processing of normal human plasma Therefore, the same general technique is employed in the collection of the whole blood and its processing until the plasma is drawn off Additional steps are required in drawing blood for packed and resuspended cells and these are stated in sub sections 2 11, 2 12, 2 13 and 2 14 The following directions are taken from section 1 6 of the minimum requirements for unfiltered normal human plasma

"Each bleeding shall be drawn into its own receiving bottle which shall be the same container used later for separating the red cells Pooling of whole blood before separating the plasma is not permitted Immediately after bleeding the blood shall be placed in a cold chamber which shall have a temperature range of 4° to 10° C (Freezing must be avoided) If transportation of the blood before removal of the red cells, from bleeding clinic to processing laboratory, becomes necessary, it shall be transported in shipping cases provided with refrigeration sufficient only so that (*a*) if the blood has already been cooled as specified above the temperature of each individual blood will not be in excess of 10° C on arrival at the processing laboratory or (*b*) if the blood is shipped before its temperature has been reduced to 4° to 10° C, the refrigeration capacity of the shipping case shall be such as to insure continued reduction (the reduction capacity need not extend below 10° C) of the temperature of each blood during the transportation interval In either event the blood shall be placed in a cold chamber having a temperature range of 4° to 10° C immediately upon arrival at the processing laboratory and until required for further processing The blood may be removed from the cold chamber during the interval required for freeing the plasma of cells It is recommended that a cooled centrifuge or one provided with a shield be used for the separation of the red cells from the plasma "

[7] Amendment of August 20 1942

2 11 The bleeding bottle and its accessories shall have been cooled to 4° to 10° C—preferably the lower limit—prior to receiving the blood

2 12 During the bleeding the anticoagulant and the entering blood are quickly and thoroughly mixed by a swirling motion Violent shaking is to be avoided

2 13 A system of adequately identifying the blood must be used which will carry through from donor to recipient This can best be accomplished by a suitable label filled out for the donor at the control desk at the time of completing the donor's personal history card, the label and history card carrying the same identification or lot number This label accompanies the donor to the bleeding room and is applied to the bleeding bottle while it is still possible to accurately check the identity of the blood with the donor and his history card

2 14 *Because of the short dating period on packed red cells and resuspended red cells* it is essential that cells be separated from the plasma within 24 hours of the removal of the blood from the donor The collected red cells must be stored continuously, including while in transit, at 4° to 10° C, the optimum range being 4° to 6° C The optimum pH range is 6 7 to 7 0 which the anticoagulant and preserving fluids are designed to maintain

2 2 *Final container for packed cells*—Packed red cells represent the mixture of red blood cells and residual citrated plasma remaining in the original bleeding bottle after the removal of the supernatant plasma The original bleeding bottle becomes the final container without the addition of any preserving fluid The bottle is fitted with a suitable sterile closure as soon as the plasma has been withdrawn, the final container label is applied, and the bottle placed in storage at 4° to 10° C—preferably 4° to 6° C Immediately before use, the packed cells may be diluted with 50 to 100 ml of sterile pyrogen free physiological solution of sodium chloride, or a solution meeting the requirements specified in section 2 4 The diluting fluid is preferably provided in a separate container by the producing laboratory in order to insure sterility and freedom from pyrogens The use of a filter during transfusion is imperative

2 3 *Final container for the resuspended cells*—The resuspended cells may be dispensed in the original bleeding bottle after the removal of the plasma provided the bottle has been fitted with a suitable closure, or in another bottle specifically adapted for this purpose If the original bleeding bottle is used, the contents of the bottle must be returned to the required volume by the addition of the sterile resuspending fluid by an aseptic technique If another bottle is used as the final container, the red cells must be transferred by an aseptic technique to this final container which already contains

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[f] Amendment of August 20 1942.

Each blood should be independently grouped by two technicians, or in a small laboratory checked by the same technician using the two methods described. A grouping procedure which assures great accuracy is to perform a single grouping of the donor's red cells against a satisfactory grouping serum. This result is checked by a second grouping test which uses the donor's serum against fresh A and B cells. A second procedure capable of giving accurate results, though believed by some to be less accurate than the foregoing in certain instances, is to determine the grouping of the donor's cells independently against two satisfactory grouping serums of different lot numbers. *A record of the tests must* be made on the donor's record card and the bottle label. This requirement applies to all donors irrespective of any previous donation, it being a record of the blood in a particular bottle and not of a person. A bleeding is not satisfactory for release if the duplicate grouping tests are not in agreement.

2.51 Another good practice, and one which is acceptable, is to securely attach a second pilot tube to the bottle. This second sample of blood accompanies the bottle of blood and is used in making compatibility tests with the recipient's blood. When this practice is followed it is advisable to provide direct ons for the proper use of the accompanying pilot blood sample.

2.52 A generally acceptable technique must be used for each grouping test and with both anti A and anti B grouping serums of acceptable avidity and potency.

2.6 *Determination of the Rh type.*—Each bottle of citrated whole blood must be typed for Rh antigen, using an anti Rh (anti D) typing serum of satisfactory avidity and potency, with a technique designed for the test serum. The result of the test must be recorded on the final container label and should also be placed on the donor's personal history card. Further subtyping of the Rh (D) *negative bloods* may be carried out, if desired, and the finding recorded on both label and history card.

2.7 *Isoagglutinin titre of group O blood.*—The isoagglutinin titres of group O bloods vary widely *and this fact becomes of importance* when group O blood is used for transfusing recipients of other groups. Consideration may be given to the addition of A and B group specific substances to each bleeding to neutralize the isoagglutinins. However, it should be *stated that this practice is not approved by* all serologists since there is evidence that the injection of these group specific substances will stimulate an increase in the agglutinins in individuals of blood groups A, B, and O. Their use is also dependent upon availability and unit cost.

the required amount of sterile resuspending fluid necessary to restore the red cells to the original blood volume It is recommended that a filter be used in the transfer tube In using this method a volume of resuspending fluid equal to one half the volume of the original blood volume is placed in the final container prior to its closure and sterilization Irrespective of which method is used the resuspending fluid should be precooled to 4° to 10° C before its use for resuspending the cells When the cells are to be transported it is advisable to fill the final container of resuspended red cells full in order to reduce the agitation of the cells while in transit

2 4 *The resuspending fluid*—For the preparation of resuspended red blood cells an acceptable resuspending fluid is one which will preserve the viability and prevent hemolysis of the red cells during the interval of the dating period provided the suspension has been stored continuously at 4° to 10° C—preferably 4° to 6° C The suspension has a pH of 6 7 to 7 0 The degree of preservation is acceptable if at the end of the dating period the survival of the red cells during the first 24 hours after transfusion is not less than 70 percent as measured by the radioactive technique or the agglutination method, and if the free hemoglobin in the overlying diluent does not exceed 25 mg /100 ml

2 41 A resuspending fluid has not been developed which will preserve the red cells as demanded by these requirements for the same interval of time as is possible with whole blood preserved in ACD solution It is therefore necessary to shorten the dating period to whatever interval the resuspending fluid will warrant

2 42 The suitability of a resuspending fluid depends upon the physical qualities mentioned in section 2 4 and upon adequate clinical trial These criteria must be applied to any solution for which recognition is desired A 10 percent solution of corn syrup meets these requirements provided the dating period is limited to not more than 10 days Corn syrup is acceptable for this purpose if it meets the specifications for Liquid Glucose USP, and if it is pyrogen free when made up as a 10 percent solution by weight in "Water for Injection," USP

2 5 *Determination of the blood group*—While packed red cells and resuspended cells from any of the four blood groups may be processed, it is probable that it becomes a practical procedure only with group O cells Each donor's blood must be classified as to blood group Blood remaining in the bleeding tube after its removal from the donor's vein is run into a suitable pilot tube This pilot tube is securely attached to the donor bottle at the time of its preparation in the laboratory It must not be detached from the bottle until the grouping tests have been completed and properly recorded

3.4 *Directions for using the cells*.—The label or an accompanying circular must give adequate directions for administration, call attention to the need for checking the label for proper blood group (if other than O cells are used), the need for careful crossmatching (see sec. 1.171, Appendix C, Minimum Requirements: Anti-Rh typing serum), the need for rigidly observing the storage temperature, the need for vigorously shaking the bottle of resuspended red cells before performing the transfusion, the *absolute* necessity of using a filter in the sterile, pyrogen free, intravenous administration equipment, the need for caution if the cells are warmed before administration, and other essential information.

3.41. If an *attached* pilot tube of the donor's blood accompanies the bottle of packed cells or resuspended cells, the cross-matching—compatibility test—with the recipient is performed on this material. In the absence of an attached pilot sample, it is recommended that the bottle be entered by inserting a very fine sterile hypodermic needle through the closure under aseptic precautions. The required amount of well mixed blood is then withdrawn with a sterile syringe which also reduces the risk of sampling error. When the latter method is used, the red cells should be used promptly since the seal has been broken.

3.42. The filter to be placed in the administration tube must not cause a critical slowing of the rate of flow of the cells to the patient. At the same time it must be capable of removing particulate matter of a size potentially dangerous to the patient. It is recommended that the filter mesh be between 100 and 200 per square inch.

3.5. *Expiration date*.—The expiration date for packed red cells and resuspended red cells shall not exceed 10 days from the date of manufacture and this only if cells have been stored continuously at 4° to 10° C.—preferably 4° to 6° C. The date of manufacture is the date of bleeding the donor.

3.6. *Shipment of packed red cells or resuspended red cells*.—In order to meet the requirements as to safety, purity, and potency as defined by the Regulations, packed red cells or resuspended red cells *must be kept continuously at 4° to 10° C*. This is also interpreted to mean while under shipment from the producing laboratory to the user. Actual shipping trials, followed by red cell viability studies, have shown the advisability of keeping this temperature at all times within the narrow range of 4° to 6° C.

2 71 An alternate method, and one which avoids this objection, is to use group O blood on the basis of the isoagglutinin titre The bleedings are placed in a *low titre* or a *high titre* category dependent upon the isoagglutinin titre A low titre indicates a blood which has an isoagglutinin titre of not more than 1 200 with the test tube centrifuge method or 1 40 with the well slide method The finding is properly recorded on the donor s record and on the bottle label Under this classification low titre blood may be given to any recipient, whereas the high titre blood is reserved for group O recipients or used for other than transfusion purposes

3 General Requirements

3 1 *The sterility test*—The requirement of aseptic technique, which is rigidly adhered to by the bleeding personnel for collecting the blood into a sterile and pyrogen free closed system and which remains continuously closed until the recipient has received the transfusion, is believed to afford greater assurance of a sterile product than can be attained by breaking the system for the removal of a sample for the sterility test In addition, a careful visual inspection of the packed red cells or resuspended red cells is made in the laboratory just prior to distribution and by the clinician before administration On both of these occasions the color and physical appearance must be consistent with the freshly drawn and prepared product

3 11 If these requirements are rigidly adhered to, a sterility test on each bottle of blood prior to distribution is not required It is recommended that the technique be checked from time to time by performing sterility tests on any remaining out dated blood For this test not less than a 5 ml sample of well mixed blood is withdrawn aseptically from the bottle and planted in a Smith fermentation tube The culture medium recommended is the National Institutes of Health thioglycollate broth medium Incubation is at 35° to 37 C for not less than 7 days

3.2 *The final container*—The final container shall be of colorless and fully transparent glass, the glass being of a quality which will not hasten the deterioration of the red cells The closure shall be adequate to maintain a contamination proof seal until the contents are to be used Once the closure seal is broken the contents of the bottle should be used promptly or discarded

3 3 *The final container label*—The label shall conform to the labeling requirements of the Regulations The recommended storage temperature should appear in a conspicuous position on the label and in distinctive printing The preferred wording is

"*Caution!* Keep continuously at 4° to 10° C"

If desired the temperature may be stated in the Fahrenheit scale, which is 39 to 50° F, or in both scales

1 21 Anti B blood grouping serum is a serum which will agglutinate human red blood cells containing B agglutinogen, i e, blood groups B and AB (subgroups A_1B and A_2B)

1 3 *Source* —Blood grouping serum is usually derived from high-titered serums of normal individuals, with or without stimulation by the injection of group specific red cells or substances, or by similar treatment of lower animals The suitability of a serum, whether liquid or dried, is determined by its ability to meet the specific tests described in these minimum requirements

2 DETAILS OF PRODUCTION

2 1 *Method of obtaining the serum* —The manner of drawing the blood shall conform to the accepted standards of aseptic surgical procedure, and the volume withdrawn, to the donor s capacity to give This is both for the protection of the donor and to avoid unnecessary contamination of the serum The apparatus used for the removal of the blood and the receiving unit shall be chemically clean and sterile

2 2 *Inactivation of blood grouping serum* —All freshly drawn serums are inactivated in a water bath at 56° C for 10 minutes before titrations are made for avidity and i ohemagglutinins

2 3 *Dilution of high titer serum* —Blood grouping serum may be diluted before dispensing provided the diluted serum meets the requirements for avidity in section 3 2 and potency in section 4 6

2 31 Diluents for serums may be a solution of sodium chloride, low titered serums of the same group specificity, or bovine or human albumin (6 to 8 percent solution) Should sodium chloride solution (preferably 1 6 to 2 0 percent) be used, the dilution should not reduce the final protein content, which is important for stability, below 25 percent of the normal protein content of the serum

2 4 *Color indicator in the finished product* —It is believed that the artificial coloring of blood grouping serum serves as a helpful guide for the clinic technician Therefore, the use of a dye is recommended, though not required

2 41 If color is added to anti A blood grouping serum, it shall be a blue dye, such as trypan blue or methylene blue, and in a concentration not less than 1 5,000

2 42 If color is added to anti B blood grouping serum, it shall be neutral acriflavin in a concentration not greater than 1 10,000, or another dye of similar color and intensity

2 43 The same color scheme is used in preparing serum to be dried

2 5 *Absence of false agglutinins or other reactions* —The finished product must not contain active agglutinins other than those specified on the label They must be free of these undesirable qualities (described briefly in sec 3 of appendix C), namely, hemolysins,

Federal Security Agency
National Institutes of Health
Bethesda 14 Md
1st revision January 20 1948.
(Reprinted May 26 1949)

11 Minimum Requirements Blood Grouping Serum

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1 The Product

1 1 *Proper name*—The proper names of the two blood grouping serums are as follows

a Anti A Blood Grouping Serum
b Anti B Blood Grouping Serum

1 2 *Definition*—Anti A blood grouping serum which will agglutinate human red blood cells containing A agglutinogen, i e, blood groups A and AB (subgroups A_1, A_2, A_3, A_1B, and A_2B)

area or larger, before 3 minutes have elapsed The time intervals stated are maximal, a serum requiring a longer interval for reaction time is not sufficiently avid

4 SPECIFIC AGGLUTININ TITER

4 1 *Preparation of serum dilutions*—Using 0 9 percent solution of sodium chloride as a diluent, prepare serial twofold dilutions over a range covering the expected titer Carry over of a higher concentration of serum to the tube of next greater dilution *must be avoided* by using a fresh pipette for each dilution, or by washing out the pipette 3 or 4 times with fresh 0 9 percent solution of sodium chloride

4 2 *Preparation of red blood cell suspensions*—Cell suspensions may be prepared from oxalated citrated or defibrinated whole blood, or from the free cells in the tube in which blood has been allowed to clot (See sec 2 of appendix C for details of selecting red cells) The blood should be drawn on the day of the test However, blood cells in plasma or serum stored at 4° to 6° C may be used within 5 days of bleeding provided that a fresh suspension be prepared each working day Prepare a 2 percent suspension in terms of packed red cells which have been washed once with and suspended in 0 9 percent solution of sodium chloride

4 3 *Performance of the test*—Place test tubes (preferably having an inside diameter of 7 to 8 mm and length of 70 to 80 mm) in a suitable rack and deliver 0 1 ml of the proper serum dilution as prepared in section 4 1 to each tube Add 0 1 ml of the appropriate red cell suspension to each tube Shake the tubes in the rack and then centrifuge the tubes at 500 to 1,000 r p m for 1 minute

4 31 Observe the tubes for the presence of agglutination over a well illuminated white surface Shake the tubes just sufficiently to dislodge the "button" of cells on the bottom of the tube

4 32 The results of the agglutination test are recorded as follows

4+ "Button" of cells on gentle shaking of tube remains in one clump

3+ "Button" of cells breaks into several large clumps

2+ "Button" of cells breaks into many small clumps of approximately equal size

1+ "Button" of cells becomes finely granular in appearance but consists of definite small clumps macroscopically These aggregates must remain visible when reexamined at least 15 minutes after agitation of the tube Any doubtful reaction should be recorded as negative The 1+ reading is the end point Macroscopic, or naked eye, observation only is required

4 4 *Test to be made on finished product*—Final agglutination titrations on the serum in the bulk are set up in triplicate using three

autoagglutinins, bacteriogenic agglutinins, and the tendency to produce rouleaux formation under the conditions obtaining in most laboratories

2 51 Into each of 3 test tubes place 0 25 ml of serum To each tube add 0 25 ml of a 2 percent suspension of group O rh (Rh negative) red cells which have been washed once with and suspended in 0 9 percent sodium chloride solution Place one tube in a water bath or warm room at 37° C for 1 hour and then room temperature for an additional 2 hours, one tube at 2° to 10° C for 1 hour and observe, then reexamine after 2 hours at room temperature, and one tube at room temperature for 3 hours The presence of agglutination, hemolysis or rouleaux formation in any of the tubes is cause for rejecting the serum

3 AVIDITY TEST

3 1 *Performance of the test*—The test is performed on a glass slide and time intervals are determined by a stop watch Agglutination is determined by *macroscopic* examination

3 11 Prepare a 10 percent suspension by volume of packed fresh red cells which have been washed once with and suspended in 0 9 percent solution of sodium chloride Separate suspensions are made for each of the specific groups and subgroups stated in section 3 2 (For the selection of red cells see sec 2 of appendix C)

3 12 Place one drop of the cell suspension on the center of a clean slide and near it an equal volume of the grouping serum under test

3 13 Accurately time and record the interval between the first rapid mixing of the two drops (over an area on the slide approximately 25 mm in diameter) and beginning agglutination

3 14 The slide should be rotated or tilted from side to side intermittently from the first mixing of cells and serum The slide should not be in contact with surfaces having a temperature above 30° C

3 2 *Degree of avidity required*—A satisfactory serum shall have the following avidity characteristics

Serum	Blood group or subgroup cell	Time in seconds beginning agglutination
Anti A	A_1	15
	A_2	30
	A_1B	30
	A_2B	45
Anti B	B	15

The clumps formed must be distinct on macroscopic or naked eye observation Some of the clumps present must be 1 sq mm in surface

standard The agglutinin titers specified in section 4 6 may be ignored when titrations are made against the standard However, the avidity of the lot under test must not exceed the time intervals given in section 3 2, rather than that possessed by the standard serum

5 3 *Turbidity*—The finished product when freshly prepared or when freshly dissolved from the dried state, shall be as free of turbidity and particulate matter as can be obtained by filtration through a bacteria excluding filter of proven quality (on aging the serum may develop a slight turbidity and a small amount of amorphous material may settle out)

5 4 *Hemoglobin content*—The finished product shall contain not more than 25 mg of hemoglobin per 100 ml

5 5 *Sterility*—The finished serum shall be sterile as indicated by sterility tests made on random final container samples Three containers shall be tested if the total is 100 or less and then 1 additional container for each 50, but not more than 10 final containers need be tested The amount to be tested from each container is not less than 0 5 ml, using National Institute of Health fluid thioglycollate medium No evidence of contamination shall appear during an observation period of not less than 7 days at 35° to 37° C

6 GENERAL REQUIREMENTS

6 1 *Preservative*—The finished product may contain as a preservative 1 6,000 phenylmercuric borate or 1 5,000 sodium ethylmercury thiosalicylate With dried serum the preservative is placed only in the accompanying diluent

6 2 *Storage*—Blood grouping serum, liquid or dried, is stored at 2° to 10° C (35 6° to 50° F) or lower during the dating period Freezing the serum does not lessen its effectiveness

6 3 *Dating*—The following dating intervals are allowed provided the storage temperature specified is observed

6 31 The expiration date is not more than 1 year after date of manufacture or 1 year after date of issue if a liquid product, or not more than 5 years if dried, and provided that storage is 2° to 10° C or lower

6 32 The date of manufacture is the date of removing the blood from the donor For dating purposes the date of manufacture may be the date of last passing a satisfactory potency test

6 33 The date of issue is not more than 1 year after date of actual manufacture or date of last passing a satisfactory potency test, provided that during the interim the product is stored at 5° C or lower

6 4 *Labeling*—The color of the label should correspond to that recommended in section 2 4, if a dye has been used The wording on the label shall meet the labeling requirements of the Regulations, including a statement of the animal source of the serum

separate sets of original dilutions The end points obtained in each titration should not vary more than one dilution (tube) from the average of the 3 tests The final titer is the average of the three end points.

4 41 Additional agglutination titrations of the product in the final container need to be performed only if the product has been processed further after being introduced into the final container

4 5 *Definition of titer and unit value*—The titer of a serum is recorded as the reciprocal of the fraction denoting the greatest dilution of serum which gives 1+ agglutination (see sec 4 32) with the proper group specific red cells Thus if the end point is 1 512 the titer is 512, and this becomes the unit value In calculating the titer the dilution caused by the addition of the red cell suspension is excluded

4 6 *Agglutinin titer required*—An acceptable blood grouping serum shall have no less than the following titers

Serum	Blood group or subgroups of cells	Minimal acceptable titer or unit value
Anti A	A_1	256
	A_2	128
	A_1B	128
	A_2B	64
Anti B	B	256

5 Specific Control Tests

5 1 *Reference standard serum*—Standard anti A and anti B serums are provided by the National Institutes of Health These are for use in preparing a laboratory working standard or for control purposes in the final agglutinin titration of the serums submitted to the Institute for release

5 2 *Values required for release*—The minimum avidity and agglutinin titer of an acceptable serum when tested by the methods described in sections 3 and 4 and without the use of a reference standard shall be as follows

a Complete specificity for the groups and subgroups involved in the product

b Avidity readings no greater than the time intervals given in section 3 3

c Agglutinin titer or unit value not less than those given in section 4 6

When titrating against the standard serum by methods described in sections 3 and 4, the agglutinin titer is at least equal to the reference

APPENDIX A

RELEASE PROTOCOL FOR ANTI A BLOOD GROUPING SERUM

1 Manufacturer .... Lot No .... Date.. ...

2 Amount of coloring added .. . Amount of preservative .... ..

3 Avidity test—Using 10 percent suspensions of the following subgroups of cells

Test cells	Beginning agglutination in seconds		Size of larger clumps at end of 3 minutes	
	Control serum	Serum under test	Control serum	Serum under test
A_1 cells				
A_2 cells				
A_1B cells				
A_2B cells				

Avidity test performed at approximately ° C

4 Agglutinin titer—Using 2 percent suspensions of the following cells

Test cells	Trial No	Greatest dilution of serum which gives 1+ reaction		Average titer of 3 trials	
		Control serum	Serum under test	Control serum	Serum under test
A_1 cells	1				
	2				
	3				
A_2 cells	1				
	2				
	3				
A_1B cells	1				
	2				
	3				
A_2B cells	1				
	2				
	3				

5 Has the test described in section 2 51 of the Minimum Requirements been performed? ...

6 Sterility test—Using not less than 0 5 ml of product. ....

7 If a dried product Moisture ... percent

(To be signed by responsible operator)

6 41 If the final container is too small to carry the full proper name as given in section 1 1, in addition to the other items required by the Regulations, the producer may choose an abbreviation of the proper name for the container label Example Anti A Grouping Serum or Anti A Serum

6 42 The final container package should include a circular outlining satisfactory test tube and slide methods for performing the blood grouping test It should also mention the avidity requirements of a good serum and other important factors involved in blood grouping

6 5 *Explanation of the potency tests*—For purposes of uniform control, it is essential that the same release tests be used in each producing laboratory Therefore, it is important that the release protocols submitted to the Biologics Control Laboratory be prepared in accordance with the tests outlined in these minimum requirements These tests are admittedly not the most sensitive tests available but are quantitative in character, thereby permitting comparisons both between lots and laboratories They are not necessarily intended as the tests of choice in the diagnostic laboratory Each producer is free to recommend the test of his choice, though he should make certain that the validity of the test has adequate support

6 6 *Requirements for release*—Complete production protocols covering each lot shall be on file in the producing laboratory as required by regulations The following should be submitted to the National Institutes of Health on each lot for release purposes

a A sample of the finished serum adequate for test purposes

b A protocol similar in form to the ones reproduced in the appendices and providing the information indicated

APPENDIX C

1 Discussion of the Principles Involved in Preparation of Anti A and Anti B Blood Grouping Serums

1 1 *Introduction*—In order to draw up the foregoing minimum requirements, some additional investigations were necessary to correlate data essential for establishing standards on which to base reliable technic for qualitative and quantitative comparison of blood grouping serums From the literature and also from unpublished studies by a group of investigators, it was clear that as long as different observers used widely varying technics there could be no uniform determination of the essential characteristics of grouping serums These characteristics are, in the main, avidity, titer, and specificity

1 11 By avidity is meant the speed and the degree of agglutination of the red blood cells being tested By titer is meant the greatest dilution of a grouping serum which will give clearly detectable agglutination Specificity is essential and will be discussed in detail later It is related to avidity in that specific agglutination must take place quickly enough and clearly for practical laboratory use Whereas avidity is not always correlated to titer, it usually is. The chief importance of a high titer is that high titered serums stand up better and deteriorate more slowly under conditions of storage and use in clinical laboratories than do low titered serums

1 2 *Titer of blood grouping serum*—Difficulties of evaluating blood grouping serums have arisen not only because many different methods have been used for determining titer and avidity, but many different technics have been used for blood grouping, often quite different from those for estimating titer and avidity

1 21 For some time workers have contended that serum should be titrated, avidity determined, etc., by the same technic as used in clinical blood grouping laboratories This is so logical that it should have been generally accepted It is generally known that when a titration is carried out on a flat slide with the end point determined macroscopically (by the naked eye), it will always be read at a lower titer than when determined in the test tube with centrifugation Likewise, when the end point is read with the aid of the low power of the microscope, the titer with either technic will be found even more elevated

1 22 There are three chief ways in use for clinical blood grouping On the flat slide, with rotation of the test mixture of cell suspension

APPENDIX B

Release Protocol for Anti B Blood Grouping Serum

1 Manufacturer ... ... Lot No . .. .. . Date ... .
2 Amount of coloring added . ... Amount of preservative .
3 Avidity test—Using 10 percent suspension of group B cells

Test cells	Beginning agglutination in seconds		Size of larger clumps at end of 3 minutes	
	Control serum	Serum under test	Control serum	Serum under test
B cells				

Avidity test performed at approximately —° C

4. Agglutinin titer—Using 2% suspension of group B cells

Test cells	Trial No	Greatest dilution of serum which gives 1+ reaction		Average titer of 3 trials	
		Control serum	Serum under test	Control serum	Serum under test
B cells	1				
	2				
	3				

5 Has the test described in section 2 51 of the Minimum Requirements been performed? .. ..
6 Sterility test—Using not less than 0 5 ml of the product
7 If a dried product Moisture .. percent

(To be signed by responsible operator)

pension of cells in 0.9 percent solution of sodium chloride. To small test tubes such as are used for Rh typing (measuring approximately 7 mm. internal diameter by 70 mm. in length) were added the same materials in the same quantities.

1.253. The well slides were shaken on the Boerner rotating machine for 10 minutes and read macroscopically immediately over an illuminated ground glass background. The degree of agglutination was read as 4 plus when one large clump, not easily broken up by gentle agitation, was found. When the clump broke up into several small ones, it was recorded 3 plus. Many small clumps, approximately equal in size, were called 2 plus, and less agglutination than this down to the highest dilution, giving fine but definitely recognizable agglutination, was called 1 plus. Questionable agglutination was considered as no agglutination. The mixtures in test tubes were centrifuged at 1,000 r. p. m. for 1 minute and then read. Agglutination in the test tubes was read and recorded in the same manner. The highest dilution causing definitely detectable (1 plus) agglutination was considered the end point and designated as the titer of the serum for the cells of the blood group, or subgroup, used. The titer was higher with the test tube centrifuge technic than with well slides (only two exceptions were observed and these with low titer serum for A_2 and A_2B cells). The ratio of well slide titer to that of test tube centrifuge technic was then determined. The ratio was not constant for different serums but titers did not vary by more than one dilution on duplicate titrations of the same serum. The ratios varied, i. e., the spread was from 1 to 2, to 1 to 16. The over all average of 61 titrations by both technics was a ratio of well slide titer to test tube titer of 1 to 6.2. The variation, or spread, in 80 percent of the titrations was in the narrower range of from 1 to 4, to 1 to 8. Therefore, if one knows the titer of a serum with one of these methods, the approximate titer with the other method can be calculated by either dividing or multiplying by 6. For absolute standardization this calculation might not be considered accurate enough and titrations by both methods may be necessary requirements. Certainly, from now on, the titer and method of titration should be plainly stated on all preparations of blood grouping serums. The titer should be given not only for cells of the main blood groups A_1 and B, but also for subgroups A_2 and A_2B.

1.254. At least this much can be deduced, if a clinical laboratory is using a flat slide or well-slide technic for determining blood groups, and the grouping serum is labeled with a low titer by test tube centrifuge titration, then the titer with either slide technic may be too low for the serum to be used with safety.

1.26. Just what the lowest permissible titers may be cannot be stated with complete assurance. But a consensus would probably come very close to the following figures:

and grouping serum at intervals, in well slides (Boerner) with continuous rotation on a rotating machine (Boerner) for 10 minutes with immediate reading of the tests, or in small test tubes with low speed centrifugation (about 500 to 1,000 r p m) for 1 or 2 minutes, and immediate macroscopic reading

1 23 By way of comment, it has been found that the same mixtures on flat slides or in well slides will give the same titers at the end of 10 minutes However, if the period of observation is lengthened, the end point will usually be found at a somewhat higher level Once this had been determined, to simplify the work all slide tests were performed in Boerner well slides with reading immediately after 10 minutes on the Boerner shaking machine (120 oscillations per minute)

1 24 The interesting observations of others with the test tube titration technic have been confirmed Once the mixtures have been placed in the test tubes, the subsequent centrifugation causes immediate agglutination and gives a sharp end point which is not appreciably changed by preliminary or additional incubation in a water bath for an hour, or on standing (This does not apply to anti Rh serums)

1 25 It is obvious that a grouping serum might be satisfactory for routine laboratory use with a sensitive test tube centrifuge technic, and be less satisfactory and unreliable when used with a less sensitive slide technic One solution to this problem would be to require titration and standardization of all test serums by both well slide and test tube technics Another solution would be possible if by comparison a ratio could be found to exist for the titers, etc, found by the two technics If this should prove to be so, then the results with one technic could be calculated in terms of the other Such a comparison was made with about 50 different test serums Macroscopic readings were used for each technic, not only because it is simpler and does not require a microscope, but because experience has shown that the end point by microscope is not as sharp and definite, and gives higher readings which would be confusing when interpreted by a technician using macroscopic readings.

1 251 Both anti A and anti B blood grouping serums were tested Anti A serums were tested with A_1, A, and A_2B cells Some of the serums were liquid, some had been diluted because their original high titer and avidity permitted this, and some had been dried *in vacuo* from the frozen state Most were human serums but some were from animal sources

1 252 The titer was determined by making a progressive series of dilutions of serum in physiological saline in a geometrical series, 1 to 2, 4, 8, 16, to 1024 In the depressions in the well slides were placed one drop of 0 9 percent solution of sodium chloride, one drop of serum dilution, and one drop of a 2 percent (in terms of blood sediment) sus

percent sodium chloride solution The blood group of each sample of blood should have been determined previously with a high titer and serum of proved specificity The cell suspensions to be prepared should be of blood groups A_1, A_2, A_2B, B, and O Using the well slide or test tube centrifuge technic (see the minimum requirements for details), set up blood grouping tests with each serum and all of these cell suspensions Anti A serum should cause agglutination of A_1, A_2, and A B cells but not of group B and group O cells. Anti B serum should cause agglutination of A_2B and B cells but not of A_1 A_2, and O cells With the second technic, prepare 2 percent suspensions as above, but the cells in each instance should be prepared in serum from the same sample of blood from which the cells are obtained Set up blood grouping tests as just described The results of these tests should be the same as with the saline suspension of cells.

1 32 With some lower animal blood grouping serums, nonspecific results have been obtained with the cells suspended in their own serum This is caused by incompletely absorbed anti human agglutinins in the animal serums in the presence of a sufficient amount of human serum The practical significance of this is that if heavy suspensions of blood in saline are accidentally used for blood grouping tests the additional amount of human serum present will cause non specific agglutination and serious errors in determination of the blood groups

1 33 From this discussion it is clear that anti-A serums should agglutinate cells of subgroups A_1, A_2, A_1B, and A B Also, they should pick up the cells of the rare individuals belonging to subgroups A_3 and A_3B Fortunately there are no definite subgroups of B

1 34 Whereas this discussion is concerned with grouping serums, it would not be complete without calling attention to the absolute necessity of proving the blood group by setting up test tube centrifuge tests with every individual s serum in two tubes, one containing a 2 percent suspension of known, fresh group A cells, and the other containing group B cells Thereby the expected agglutinins corresponding to each blood group will be found—both anti A and anti B for group O, anti B for group A, anti A for group B, and no agglutinins for A_1B, A_2B, and A_3B This will not only check the accuracy of the direct grouping, but will catch any dangerous weakening of avidity, or loss of specificity from bacterial contamination or other reasons. It will help detect, also, any errors in records and reports Only very rarely expected agglutinins will be so weak that they will have to be demonstrated by adding a drop of homologous serum, or group AB serum, or 20 percent purified human or bovine albumin solution to the test tube test.

Blood grouping serum	Well-slide technic	Test tube centrifuge technic
Anti A		
A_1 cells	1 to 80–100	1 to 480–600
A_2 cells	1 to 40–60	1 to 240–360
A_2B cells	1 to 20–30	1 to 120–180
Anti B		
B cells	1 to 40–60	1 to 240–360

1 27 As a matter of fact, the best anti A blood grouping serums produced today cause practically as rapid and massive agglutination of A_2 cells as of A_1 With poor anti A serum one can usually pick out A_2 cells by the smallness of the clumps and the slowness of agglutination Formerly this was quite generally true But today with anti A serum produced with stimulation of anti A agglutinins by injecting very small quantities of A substance or pooled plasma into individuals belonging to group B, A_2 cells are agglutinated practically as well as A_1 cells Also, the anti A_2 titer is almost as high as for A_1 cells However, even the best "stimulated" serums, although they agglutinate A B cells clearly, are not quite as active for these as for A cells This additional agglutinating activity is an advantage, not a disadvantage Other methods are available for picking out A_2 and A B What is of greatest importance is that A , either alone or combined with B, should not be missed Otherwise A_2 individuals might be classified as O, and A_2B as B, if the only grouping test used was with anti A and anti B serums and the individual's cells This will be discussed later

1 28 On the basis of titration one can specify an agglutinating unit *Anti B serum will contain as many units as the numeral indicating the highest dilution which will cause minimal detectable agglutination with the technic used for titration* Thus, if the end point is 1 40 with the well slide technic, the undiluted serum will contain 40 units for this technic and probably about 240 units by the test tube centrifuge technic Also, anti A serum may contain 80 units for A_1 cells, 60 units for A_2 cells, and 30 units for A_2B cells by the well slide technic

1 3 *Specificity of blood grouping serum*—The blood grouping serums must be completely specific for cells of the blood group or groups they are supposed to agglutinate and none other They should contain neither general anti human agglutinins (for human cells of other blood groups) nor anti O agglutinins such as are present in many animal serums

1 31 The specificity of each serum should be determined by two technics in order to assure accuracy With the one method, a 2 percent suspension of once washed, packed cells is prepared in 0 9

a The protein content of the testing serum must not be reduced below 25 percent of the normal value for serum, since this factor appears to lend stability to the grouping serums Thus low titered serums of the same group specificity, or albumin solutions (6 to 8 percent) should be used with or without saline diluent

b It appears that a final concentration of 1 4 percent of sodium chloride is optimal for avidity Thus serum should be diluted with 1 6 to 2 0 percent solution of sodium chloride to obtain the optimal concentration in serum cell mixtures

1 46 Blood grouping serums keep better in the frozen state and even the small bottle of serum used for daily routine testing should be kept frozen as much of the time as possible This also will keep down loss of potency by accidental bacterial contamination It is also well recognized that a serum *diluted with 0 9 percent solution of* sodium chloride deteriorates more rapidly than undiluted serum or serum diluted with specific human serum

1 5 *Direct compatibility tests*—In addition to determining the compatibility of donor and recipient blood by blood grouping with control serums direct matching or compatibility tests are also required. Compatibility tests are an additional check on possible errors in blood grouping The most recently described tests also will discover intra group and atypical agglutinins which will be missed when only the usual methods of blood grouping are used It is now realized that it is of great importance to discover these incompatibilities to avoid performing transfusions which would cause severe or even fatal reactions

1 51 Compatibility tests in the past have been carried out by making two preparations one a mixture of recipient serum with a 2 percent saline suspension of donor cells, and the other with donor serum and recipient cells A drop of each is mixed on a flat slide and then observed for agglutination This is the least sensitive and least accurate method we have Tests in well slides or in test tubes with centrifugation and observation under the low power microscope are much better technics

1 52 The newest methods avoid cell suspensions in saline and utilize whole oxalated blood and oxalated plasma Agglutinins will be discovered that are missed by the other technics *Blood from* both donor and recipient is collected in dry test tubes containing an amount of dry oxalate, sufficient to prevent coagulation A portion of the sample is placed in a separate tube, centrifuged, and the supernatant plasma withdrawn The two types of mixtures are then made using, however, whole blood and oxalated plasma Even on slides this is a better test than the other, especially if the slides are observed for agglutination on top of a warm, illuminated box The test tube

14 *Avidity of blood grouping serum*—The avidity of test serums must be good, i e, they must cause agglutination rapidly and in large or easily detectable clumps It is obvious that the avidity of anti A serum must be determined not only with subgroup A_1 cells but also with subgroups A_2 and A_2B cells

141 Since most laboratories carry out blood grouping tests on flat slides, avidity tests have been performed with this technique It is generally conceded, however, that the flat slide technique is the least sensitive method and the one most likely to give erroneous results This should be stated clearly in any discussion on blood grouping, and all laboratories should be urged to change to a better method (well slide or test tube) With the flat slide or well slide techniques, the test should be observed for 10 minutes before reading it With the preparation spread out on the flat slide, evaporation at room temperature may cause the preparation to dry out before 10 minutes and make interpretation difficult or impossible This does not happen with the well slide technic

142 The flat slide technic is, briefly as follows One determines with a stop watch the time in seconds of beginning macroscopic agglutination On a slide are placed, side by side, a drop of *10 percent cell suspension* in physiological saline and a drop of test serum The two drops are then rapidly mixed and spread out over a circular area not quite 2 5 cm (1 inch) in diameter The stop watch is started at the same time as mixing The slide is then rotated, or inclined, from side to side to keep the mixture moving and is observed over an illuminated ground glass plate The time is observed for beginning agglutination and the size of the clumps is noted at a stated interval

143 In general test serums should show beginning agglutination for subgroup A_1 and for group B cells in not more than 15 seconds The interval may be longer for subgroups A and A_2B cells The clumps should be 1 sq mm in surface area after 3 minutes of continuous rotation

144 Although slide testing is not recommended for blood grouping the slide avidity test together with titration, gives reliable information of the dependability of the test serums Avidity and titer are not always correlated—at times a high titered serum may have low avidity, and the reverse may also occur The lack of correlation, when it is present, is not clearly understood

145 High titered blood grouping serums may be prepared by injecting group specific cells or substances into a suitable donor These serums, in general are considered best for use as diagnostic reagents Sometimes they are so potent that they can stand "cutting" or dilution, and at times are even improved by dilution Two factors must be considered in making such dilutions

titrations The method recommended for selecting A_2 and A_2B cells depends upon the preparation of an anti A serum which has had agglutinins for A_1 cells removed by absorption with known A_1 cells The following procedure is recommended

2 211 From a series of red blood cells obtained from 10 or more group A individuals, prepare 2 percent suspensions by volume of packed cells in 0 9 percent solution of sodium chloride and set up titrations using a potent anti A serum (titer 256 or higher) Cells agglutinated to full titer, or one tube below titer, will represent A_1 cells Those agglutinated by the stronger concentrations but not by the more dilute serum may be of subgroup A Select the cells which give the lowest titration value for absorbing A_2 agglutinins from the anti A serum

2 212 Mix the serum with ¼ its volume of packed freshly drawn red cells, known to give definitely lower titers as described above Allow the mixture to stand for ½ hour to 1 hour at room temperature, then centrifuge for 2 minutes at 1,000 r p m and remove the supernatant serum The serum should fail to agglutinate a fresh suspension of the same cells with which it was absorbed, but will agglutinate A_1 and A_1B cells The serum should fail to agglutinate other cells giving weak reactions with potent anti A serums Thus cells agglutinated by absorbed anti A serum will be of the A_1 subgroup Red cells known to be group A but which are not agglutinated by the absorbed anti A serum will belong to group A Likewise, A_1B cells will be agglutinated while A_2B will not be agglutinated by the absorbed serum

3 Undesirable Qualities in Blood Grouping Serums

3 1 *Isohemolysins* —In the presence of complement hemolysis, isohemolysins will occur when blood grouping serum is set up with its specific agglutinogen Such a reaction may lead to error when fresh serum is used in blood grouping tests Fresh serum should be inactivated by heating at 56° C for 10 minutes in order to destroy complement Serum which has been stored for several weeks or longer, or which has been diluted four to five times its volume with saline, will be free of the danger of hemolysis interfering with blood grouping tests

3 2 *Rouleaux formation* —The tendency for a serum to produce rouleaux formation or pseudoagglutination renders it unsatisfactory for use as a diagnostic agent The arrangement of the red cells with their flat surfaces in contact in the presence of serum is a familiar finding in certain infections and conditions of man, and is the basis for the widely used sedimentation rate test in clinical medicine This property of serum may be distinguished from true agglutination by the ease with which the rouleaux are broken up by agitation or by the

technic is a better one Probably the best method, at present, is to work with 2 percent suspensions of cells in their own oxalated plasma Make the mixtures in small test tubes such as used for Rh typing (approximately 7 mm internal diameter by 70 mm in length) Incubate in a water bath at body temperature for 1 hour, and centrifuge at 500 to 1,000 r p m for 1 minute Then gently roll the tubes to dislodge the cells from the bottom of the tubes (do not shake), and examine by naked eye and low power microscope, in the small test tube, for any agglutination

1 521 This technic is so sensitive and accurate that it should be required for all crossmatching Many, possibly all, transfusions which would cause reactions by specific incompatibility may be avoided by this comparatively simple test This technic is very sensitive in detecting weak anti A and anti B agglutinins, intra group agglutinins, Rh antibodies, and probably will detect other atypical agglutinins

2 Technical Considerations Involved in Blood Grouping Serums

2 1 *Preparation of red blood cell suspensions*—Blood drawn by venipuncture and mixed in a tube with dry oxalate is the most useful blood preparation for blood grouping tests Test tubes may be prepared by adding a solution of potassium and ammonium oxalate (one drop or 0 05 ml for each 2 ml of blood to be drawn) to a clean test tube which is then dried in an incubator or oven The oxalate solution may be prepared by dissolving 6 gm of ammonium oxalate and 4 gm of potassium oxalate in 100 ml of distilled water

2 11 To prepare suspensions of red cells in saline (or albumin or plasma), freshly drawn cells should be placed in a graduated centrifuge tube and spun until the red cells are firmly packed in the tip of the tube (2,000 r p m for 10 minutes) The supernatant fluid should be poured off and the suspending fluid added, so that the volume of packed red cells will represent the desired percent of the final mixture

2 111 As an example To prepare a 2 percent suspension of red blood cells in saline, centrifuge a heavy suspension of cells in saline Assuming that the packed cells occupy the graduated centrifuge tube to the 0 3 ml mark, add saline to the 15 ml mark and resuspend the cells

2 2 *Selection of the test red blood cells*—Variation in the agglutinability of red blood cells of subgroups A_1, A_2, A_1B, and A_2B by anti A blood grouping may be recognized by (1) the comparative speed of agglutination of such cells, (2) the differences in titer of the serum when measured by the subgroups of cells, (3) the size of the clumps formed by the action of the serum on the cells

2 21 Obviously, variations in the sensitivity of cells used for testing anti A serum will affect values obtained by avidity and potency

[Federal Security Agency
National Institutes of Health
Bethesda 14 Md
2d revision May 25 1949]

IV Minimum Requirements Anti-Rh Typing Serums

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34" — 5——5

addition of saline to the serum cell mixture It will be noted that rouleaux formation is more apt to occur when the flat slide technic of testing is employed, due to the concentration of serum as a result of more rapid evaporation Serum which produces rouleaux formation should not be used in blood grouping tests

3 3 *Autoagglutination* —Serum to be used in blood grouping tests should be free of so called autoagglutinins As a rule, autoagglutination is apparent when serum and red cells from the same individual are placed at temperatures lower than room temperature (preferably 2° to 6° C), agglutination is weakened or abolished at 37° C Occasionally serums are found which agglutinate autologous cells at room temperature These reactions are based on the presence of an absorbable agglutinin in the serum and an agglutinogen in human red cells It acts on all human red cells irrespective of group or type, Obviously, serum containing autoagglutinins which are active at room temperatures cannot be used as blood grouping serum

3 4 *Bacteriogenic agglutination* —It is well known that red-cell suspensions after being contaminated by the growth of certain bacteria may become agglutinable by serum, regardless of the group or type of cells This phenomenon may be avoided by the use of fresh blood cell suspensions

3 41 Contamination of serum by the growth of certain bacteria may also lead to false positive agglutination reactions Careful, sterile technic in entering serum containers and the presence of an effective preservative will prevent this undesirable reaction

1.24 Anti-rh' typing serum agglutinates all human red blood cells containing rh' factor (types Rh_0', $Rh_0'Rh_0''$, rh', and rh' rh''), it does not agglutinate cells containing only Rh_0 and rh'', nor rh type cells

1.25 Anti-rh'' typing serum agglutinates all human red blood cells containing rh'' factor (types Rh_0'', $Rh_0'Rh_0''$, rh'', and rh' rh''), it does not agglutinate cells containing only Rh_0 and rh', nor rh type cells.

1.26 Anti-Rh typing serums must not contain agglutinins for A or B agglutinogen, and must be free of atypical agglutinins as anti-O, anti-P, etc. If such agglutinins are present in the original serum, they must be removed by absorption with appropriate red blood cells, or their action neutralized by the addition of group specific substances as needed

1.3 *Source*.—Anti-Rh typing serums are derived from women who have developed Rh antibodies as a result of sensitization to fetal Rh-positive red cells, and from individuals of either sex who have become sensitized as a result of *repeated* transfusions or injections of Rh-positive blood

1.31 Anti-Rh serums of human origin are of two general classes, depending upon the immune response in different individuals to exposure to Rh factor In one instance Rh antibodies may be found in the serum of sensitized individuals which will cause agglutination of Rh-positive cells suspended in physiologic saline This type of antibody is designated by various workers in the field as "complete," "heat labile," "agglutinin," "bivalent," "early immune," etc In other instances and more frequently, Rh antibodies resulting from sensitization to Rh-positive cells will not agglutinate (or only weakly agglutinate) saline suspensions of Rh-positive cells but will agglutinate Rh-positive cells in the presence of a sufficient amount of protein (serum or albumin) This type of antibody is designated by various workers in the field as "blocking," "univalent," "incomplete," "heat stable," "glutinin," "hyperimmune," etc The term "blocking" antibody will be used in these minimum requirements

2 DETAILS OF PRODUCTION

2.1 *Method of obtaining the serum*.—The manner of drawing the blood shall conform to the accepted standards of aseptic surgical procedure, and the volume withdrawn to the donor's capacity to give This is both for the protection of the donor and to avoid unnecessary contamination of the serum The apparatus used for the removal of the blood and the receiving unit shall be chemically clean and sterile

2.2 *Inactivation of the serum*.—Anti-Rh typing serums are not inactivated by heating prior to use

1 THE PRODUCT

1 1 *Proper name* —The proper name of a diagnostic serum containing one or more of the Rh antibodies shall be "Anti Rh Typing Serum" In order to fully identify the serum, the type designation of the serum is given immediately below the proper name This may appear in type of lesser point size and face (See sec 7 4 for additional labeling requirements)

1 11 The Rh type designation is given in both the Wiener and Fisher Race classifications and in the following arrangement

Anti Rh (Anti D)
Anti Rh (Anti C+D)
Anti Rh (Anti D+E)
Anti Rh Rh (Anti C+D+E)
Anti rh (Anti C)
Anti rh (Anti E)
Anti rh rh (Anti C+E)

Note —The use of the + sign is optional.

The processing laboratory may place the approximate percentage distribution of the type involved on the label, if so desired

1 12 The Hr type designation is given in both the Wiener and Fisher Race classifications and in the following arrangement

Anti Hr (Anti d)
Anti hr (Anti c)
Anti hr (Anti e)

1 13 In the preparation of these minimum requirements the nomenclature of only one system of classification of the Rh factor has been used This has been prompted by a desire for brevity and to make the text less involved The Rh system has been used because it appears more generally in published literature The scientific staff in each producing laboratory will be acquainted with both systems required to be stated on the label (see sec 1 1)

1 2 *Definition* —The anti Rh typing serums are defined as follows

1 21 Anti Rh_o typing serum is specific for the Rh factor It agglutinates all human red blood cells containing Rh_o factor (types Rh_o, Rh ', Rh_o' , Rh_o Rh_o') , it does not agglutinate cells containing only rh' and rh'', nor rh (Rh negative) cells

1 22 Anti Rh typing serum agglutinates all human red blood cells containing Rh and rh factors (types Rh , Rh_o , Rh_o'', Rh ' Rh '', rh', and rh rh'') , it does not agglutinate cells containing rh'' factor alone, nor rh type cells.

1 23 Anti Rh_o typing serum agglutinates all human red blood cells containing Rh and rh factors (types Rh , Rh ', Rh_o'', Rh ' Rh_o'', rh ', and rh' rh'') , it does not agglutinate cells containing rh' factor alone, nor rh type cells

cells in plasma or serum stored at 4° to 6° C may be used within 5 days of bleeding, provided that a fresh suspension be prepared each working day Prepare a 2 percent suspension in terms of packed red cells which have been washed once with, and suspended in, 0 9 percent solution of sodium chloride

3 3 *Performance of the test*—Place test tubes in a suitable rack and deliver 0 1 ml of the proper serum dilution, as prepared in section 3 1, to each tube Add 0 1 ml of the appropriate red cell suspension to each tube Mix the contents of the tubes by gently shaking the rack

3 31 The tubes containing serum dilutions and cells are placed in a water bath at 37° C without disturbance for 60 minutes

3 32 Centrifuge the tubes at a speed and interval which will cause all cells to be formed into a firm button Each centrifuge should be calibrated in advance as to r p m and interval required (2 minutes at 1 000 r p m in an angle centrifuge having a radius of 4 to 8 cm is approximately correct) Each run should include a control tube containing 0 1 ml of the appropriate red cell suspension (see 3 2) plus 0 1 ml of the same diluent used for diluting the serum under test *After centrifugation the cells in this tube should give a negative* reaction when examined as described in section 3 33

3 33 Remove tubes from centrifuge and examine each tube by carefully holding tube in the horizontal position and gently rotating it to mix the cells and supernatant liquid

3 34 Macroscopic examination of the gently resuspended cells is required to determine the end point The results of the agglutination tests are recorded as follows

4+ "Button" of cells on gentle agitation remains in one clump

3+ "Button" of cells breaks into several large clumps

2+ "Button" of cells breaks into many small clumps of approximately equal size

1+ "Button" of cells becomes finely granular in appearance but consists of definite, small clumps macroscopically These aggregates must remain visible when reexamined at least 15 minutes after agitation of the tube Any doubtful reaction should be recorded as negative The 1+ reading is the end point

3 35 Agglutination titrations are set up using at least 4 red cell suspensions of known Rh types for which there may be agglutinins present in the serum As examples, in testing anti Rh_o typing serum cells of 2 Rh ′ and 2 Rh ″ rh′ and rh ′ individuals are recommended, for testing Anti Rh_o′ Serum cells from Rh_o′, Rh ″ and rh′ and rh″ individuals should be tested (see sec 2, appendix C, *for selection of* cells) In addition, cells from a known rh (Rh negative) type individual are used as a negative control

2 3 *Dilution of high titer serum*.—Anti Rh typing serum may be diluted prior to dispensing, provided the finished diluted serum meets the requirements specified in section 6 2

2 31 Diluents for serum are of the following two classes

a For tests in which saline suspensions of red cells will be agglutinated by anti Rh agglutinins 1 6 to 2 0 percent solution of sodium chloride may be used, provided the protein content of such diluted serum is not less than 25 percent of the normal protein content of the serum and the final salt content is not more than 1 4 percent Also, this class of anti Rh serum may be diluted with albumin (6 to 8 percent solution)

b Serums containing blocking antibodies and intended for use in modified tests must not be diluted with saline solution They may be diluted with 20 percent albumin solutions (human or bovine) or human serum or oxalated plasma from group AB blood

2 32 Albumin solutions and serum used as diluents must be free of group isohemagglutinins and nonspecific agglutinins

2 4 *Absence of false agglutinins or other reactions*.—The finished product must not contain active agglutinins other than those specified on the label They must be free of the undesirable qualities (described briefly in sec. 3 of appendix C), namely, hemolysis, autoagglutinins, and bacteriogenic agglutinins

2 41 Into each of 3 test tubes place 0 25 ml of serum To each tube add 0 25 ml of a 2 percent suspension of group O rh (Rh negative) red cells which have been washed once with and suspended in 0 9 percent sodium chloride solution Place one tube in a water bath or warm room at 37° C for 1 hour and then room temperature for an additional 2 hours, one tube at 2° to 10° C for 1 hour (observe and reexamine after 2 hours at room temperature), and one tube at room temperature for 3 hours The presence of agglutination, hemolysis, or rouleaux formation in any of the tubes is sufficient to reject the serum

3 AGGLUTININ TITER OF SERUM BY THE TEST TUBE TEST WITH SALINE SUSPENSIONS OF RED CELLS

3 1 *Preparation of serum dilution*.—Using 0 9 percent solution of sodium chloride as a diluent, prepare serial twofold dilutions over a range covering the expected titer Carry over of a higher concentration of serum to the tube of next greater dilution *must be avoided* by using a fresh pipette for each dilution, or by washing out the pipette three or four times with fresh 0 9 percent solution of sodium chloride

3 2 *Preparation of red blood cell suspensions*.—Cell suspensions may be prepared from oxalated, citrated, or defibrinated whole blood, or from the free cells in the tube in which blood has been allowed to clot (See sec 2 of appendix C for details of selecting red cells) The blood should be drawn on the day of the test. However, blood

must have produced clumps at least 1 sq mm in surface area when tested against red blood cells specified in section 3 35

5 Agglutinin Titer of Serum Containing Blocking Antibodies

5 1 *Preparation of serum dilutions*—Dilutions of serum containing blocking antibodies are prepared as outlined in section 3 1 using a 20 percent solution of albumin (human or bovine) or plasma or serum from group AB blood (not containing irregular antibodies as anti A etc) in place of saline as a diluent

5 2 *Preparation of red cell suspension*—Fresh red blood cells of the proper types for testing the serum are packed by centrifuging, the supernatant oxalated plasma withdrawn, and the unwashed cells are made up to 2 percent suspensions in their own plasma or serum, 15 percent albumin solution, or plasma or serum from group AB blood

5 3 *Performance of the test*—The agglutination test is performed as outlined in section 3 3 and is read as in 3 31

5 4 *Agglutinin titer required of serum containing blocking antibodies*—Requirements for serum containing blocking antibodies are identical with those expressed in section 3 6, namely, 1+ reaction with the requisite cells when the serum is diluted not less than 1 32

5a Agglutinin Titer of Serum Containing Blocking Antibodies (alternate test method)

5 1a *Preparation of serum dilutions*—The serum under test should be diluted so that the possibility of "carryover" of serum from a lower to a higher dilution is absolutely eliminated Prepare a series of tubes covering the estimated titer of the serum, for example, into separate test tubes place 1 5 ml, 3 1 ml, and 6 3 ml of 20 percent bovine albumin Place 0 1 ml of the serum to be tested into each tube and mix the contents, using a separate pipette for each tube Similar dilutions are prepared with the reference standard serum

5 2a *Preparation of red cell suspension*—Oxalated fresh whole blood or 40 percent suspensions of free cells in the serum of clotted blood of the proper types for testing the serum is selected (See section 3 35)

5 3a *Performance of the test*—On the surfaces of three glass microscope slides, place 0 05 ml of serum from each of the tubes prepared as in section 5 1a Using a pipette delivering the same volume per drop, place 0 1 ml of oxalated whole blood on each slide

5 31a At the first *mixing of serum and cells, start a stop watch* Carefully mix serum and cells and spread the mixture over an area approximately 20 mm x 40 mm on the slide, shaping the liquid into a rectangular area Place the slide on an illuminated surface, the temperature of which should be at least 37° C and not exceed 47° C

3.4 *Test to be made on finished product*.—Final agglutination titrations on the serum in bulk are set up as described in section 3.3, using the cells required in section 3.35. The agglutinin titer is the average value obtained in tests with the prescribed cells.

3.41 Additional agglutination titrations of the finished product in final container need be performed only if the product has been further processed after being introduced into the final container.

3.5 *Definition of titer and unit value*.—The titer of an anti-Rh typing serum is recorded as the reciprocal of the fraction denoting the greatest dilution of a serum which gives a 1+ reaction (see sec. 2.34) with the proper Rh positive cells. Thus if the end point is 1:128 the titer is 128 and this becomes the unit value. In calculating the titer the dilution caused by the addition of the red cell suspension is excluded.

3.6 *Agglutinin titer required*.—An acceptable anti-Rh typing serum shall give a 1+ reaction with the requisite cells when diluted not less than 1:32.

4. Avidity Test for Serum Containing Blocking Antibodies

4.1 *Slide test serum*.—Since these testing serums are intended chiefly for performing tests on flat slides, avidity requirements are necessary in addition to agglutinin titrations.

4.2 *Performance of the test*.—The test is performed on a glass microscope slide placed on a warm, illuminated surface. Time intervals are determined by stop watch. Agglutination is determined by macroscopic examination.

4.21 Prepare 40 percent suspension of red blood cells (from whole blood oxalated with dry oxalate) of the requisite Rh types (see sec. 3.35) in their own plasma or serum or in plasma or serum from group AB blood. The red cells of clotted blood may be sedimented from the serum and 40 percent suspensions may be made in the suspending fluids as above.

4.22 Place two drops of the cell suspension on the center of a clean microscope slide, and near it an amount of the anti-Rh serum under test equal to one half the volume of the cell suspension.

4.23 Accurately time and record the interval between the first rapid mixing of cells and serum (over an area on the slide approximately 20 mm. x 40 mm.) and beginning agglutination.

4.24 The slide should be placed on an illuminated glass surface which is warmed by the light source (temperature not to exceed 47° C.) and should be rocked from side to side continuously for 2 minutes. At the end of this time, the size of clumps present should be noted.

4.3 *Degree of avidity required*.—A satisfactory serum must show beginning agglutination within 60 seconds, and at the end of 2 minutes

through a bacteria excluding filter of proven quality (On aging, the serum may develop a slight turbidity and a small amount of amorphous material may settle out)

6 4 *Hemoglobin content*—The finished product shall contain not more than 25 mg of hemoglobin per 100 ml

6 5 *Sterility*—The finished serum shall be sterile, as indicated by sterility tests made on random final container samples Three containers shall be tested if the total is 100 or less and then 1 additional container for each 50, but not more than 10 final containers need be tested The amount to be tested from each container is not less than 0 5 ml, using National Institutes of Health fluid thioglycollate medium No evidence of contamination shall appear during an observation period of not less than 7 days at 35° to 37° C

7 General Requirements

7 1 *Preservative*—Anti Rh typing serum is dispensed without a preservative until such time as a non denaturing preservative becomes available

7 2 *Storage*—Anti Rh typing serum, liquid or dried, is stored at 2° to 10° C (35 6° to 50° F) or lower during the dating period Freezing the serum does not lessen its effectiveness

7 3 *Dating*—The following dating intervals are allowed provided the storage temperature specified is observed

7 31 The expiration date is not more than 1 year after date of manufacture or 1 year after date of issue, if a liquid product, or not more than 5 years, if dried, and provided that storage is 2° to 10° C or lower

7 32 The date of manufacture is the date of removing the blood from the donor For dating purposes the date of manufacture may be the date of last passing a satisfactory potency test

7 33 The date of issue is not more than 1 year after date of manufacture or date of last passing a satisfactory potency test provided that during the interim the product is stored at 5° C or lower

7 4 *Labeling*—The wording on the label meets the labeling requirements as given in the Regulations, including a statement of the animal source of the serum The label also carries a statement indicating the test method which must be employed with the serum

7 41 If the final container is too small to carry the full proper name as given in section 1 1, in addition to the other items required by the Regulations, it may be abbreviated to Anti Rh_o "(Anti D) Serum" with similar abbreviations for the other types

7 42 The final container package should include a circular outlining a satisfactory method for use in performing an Rh typing test It should also briefly discuss other matters which are essential to the proper use of the serum

5 32a At 10 second intervals tilt the slide so that most of the liquid accumulates at one end of the slide, returning the slide then to a horizontal position Alternate the direction of flow of the liquid each 10 seconds

5 33a Observe and record the degree of clumping at the end of 2 minutes The central area of the reaction should be carefully inspected for clearness of the background fluid

5 34a The end point is the greatest dilution of serum giving definite macroscopic clumping of cells with clear, colorless background fluid 2 minutes after the first mixing of the serum and cells

5 4a *Agglutinin titer required of serum containing blocking antibodies*—An acceptable anti Rh serum containing blocking antibodies shall agglutinate specific cells when diluted not less than 1 32 under the conditions described in sections 5 1a to 5 3a When tested with the reference standard serum, the titer shall be at least equal to the reference standard

6 Control Tests

6 1 *Reference standard serum*—Standard anti Rh serums will be provided by the National Institutes of Health, but their availability will be dependent upon the adequacy of the serum supply An anti Rh_o serum is presently available This serum is for use in preparing a laboratory working standard or for control purposes in the final titration of the serum submitted to the Institute for release

6 2 *Values required for release*—The avidity of an acceptable serum when tested by the method described in section 4 and its agglutinin titer when tested by the method described in sections 5 and 5a is specified in this section Similarly the agglutinin titer of serum by the test tube test with saline suspension of red cells is also specified as follows

a Complete specificity for the types and subtypes involved in the product. (See sec 2 2 of appendix C for specificity requirements)

b Agglutinin titer or unit value not less than that given in sections 3 6, 5 4, and 5 4a

c Avidity reading of a serum containing blocking antibodies no greater than the intervals given in section 4 3

When titrating against the standard serum, if one is provided, by methods described in sections 3, 4, and 5, the agglutinin titer and avidity is at least equal to the reference standard. The agglutinin titers specified in sections 3 6, 5 4, and 5 4a may be ignored when titrations are made against the standard

6 3 *Turbidity*—The finished product when freshly prepared, or when freshly dissolved from the dried state, shall be as free of turbidity and particulate matter as can be obtained by filtration

APPENDIX A

RELEASE PROTOCOL FOR ANTI RH TYPING SERUM CONTAINING BLOCKING ANTIBODIES (THIS PROTOCOL IS DESIGNED FOR USE WITH ANTI RH SERUM SLIGHT CHANGES WILL MAKE IT SUITABLE FOR RECORDING TESTS ON A SERUM OF ANOTHER RH TYPE)

1 Manufacturer .... Lot No . ... Date .. ..

2 Avidity test—Using 40 percent suspension of cells of the following Rh types in own plasma serum or 20 percent albumin solution

Test cells	Beginning agglutination in seconds		Size of larger clumps at end of 2 minutes	
	Control serum	Serum under test	Control serum	Serum under test
Rh ′ (CDe)				
Rh ′ (CDe)				
Rh ″ (cDE)				
Rh ″ (cDE)				
rh (Cde)				
rh ′ (cdE)				
rh (cde)				
Avidity test performed at approximately —° C				

3 Agglutinin titer—Using 2 percent suspensions of the following Rh types of cells

Test cells	Greatest dilution giving a 1+ reaction	
	Control serum	Serum under test
Rh ′ (CDe)		
Rh (CDe)		
Rh (cDE)		
Rh (cDE)		
rh (Cde)		
rh (cdE)		
rh (cde)		
Average value obtained with Rh positive cells units		

4 Has the test described in section 2 41 of the Minimum Requirements been performed?

5 Sterility Test—Using not less than 0 5 ml of the product .. ..

6 If a dried product Moisture . percent

(To be signed by responsible operator)

7 5 *Other anti Rh typing serums* —Anti Rh typing serums of type specificities other than Rh_o, Rh_o', and Rh″ are of more limited use Until such serums become more widely available and more widely used, minimum release values will not be established Their production and testing should follow the pattern given in these minimum requirements, but each producing laboratory will be responsible for maintaining a suitable potency level *In addition to the labeling* requirements given in these minimum requirements, the statement "No U S Standard of Potency" must appear on the label

7 6 *Explanation of the potency tests* —For purposes of uniform control, it is essential that the same release tests be used in each producing laboratory Therefore, it is important that the release protocols submitted to the Biologics Control Laboratory be prepared in accordance with the tests outlined in these minimum requirements These tests are admittedly not the most sensitive tests available, but are quantitative in character, *thereby permitting comparisons* both between lots and laboratories They are not necessarily intended as the tests of choice in the diagnostic laboratory Each producer is free to recommend the test of his choice, though he should make certain that the validity of the test has adequate support

7 7 *Requirements for release* —Complete production protocols covering each lot shall be on file in the producing laboratory as required by the Regulations The following should be submitted to the National Institutes of Health on each lot for release purposes

a A sample of the finished serum adequate for test purposes

b A protocol similar in form to the one reproduced in the appendices and providing the information indicated

APPENDIX C

1 A Discussion of the Principles Involved in the Preparation of the Anti-Rh Typing Serums

1 1 *Introduction*.—It is only during recent years that the entire field concerning the Rh factor, and its importance in safe transfusions, has been developed. Workers in this field have discovered it to be a very complicated one, but, nevertheless, they have advanced knowledge with great rapidity and accuracy. The Rh factor was discovered by Landsteiner and Wiener when antibodies were found in animal serum after injection with blood from the *Macaccus rhesus* monkey. They found that agglutinins were produced which not only agglutinated rhesus red blood cells but also agglutinated the red blood cells of 85 percent of the mixed white population of this country (the percentages for ethnic groups are different). They termed this anti-Rh agglutinin (using the first two letters of the name "Rhesus") and the agglutinogen in red blood cells acted on by this agglutinin "Rh factor." Individuals whose red blood cells were agglutinated by anti-Rh serum were called Rh positive (now called Rh_0), and those whose cells were not agglutinated were Rh negative. That this discovery was of clinical importance was shown when the atypical agglutinin described by Levine and Stetson, in 1939, proved to be anti-Rh in specificity and later that Rh negative individuals become sensitized to Rh positive cells under certain conditions.

1 1 1 One or more transfusions, or injections, of Rh positive (having one or more factors, Rh_0, rh′, or rh″) blood may sensitize a recipient who is Rh negative (rh). A subsequent transfusion of Rh positive blood may cause a serious reaction. If an Rh negative woman becomes pregnant and the fetus is Rh positive (having inherited this factor from the father), she may become sensitized to the Rh factor. It may require several pregnancies for her to become sufficiently sensitized to reach a dangerous level, unless she has previously received a transfusion of Rh positive blood. The Rh antibodies of the mother may pass across the placenta into the blood stream of the fetus and cause passive sensitization of the fetus with destruction of its red blood cells, causing severe anemia and the entire symptom complex of erythroblastosis fetalis or hemolytic disease of the newborn. Such a fetus may die *in utero*, or if born alive may require immediate or early transfusion of Rh negative blood.

APPENDIX B

RELEASE PROTOCOL FOR ANTI RH TYPING SERUM TO BE USED WITH SALINE SUSPENSIONS OF CELLS (THIS PROTOCOL IS DESIGNED FOR USE WITH ANTI Rh_0 SERUM SLIGHT CHANGES WILL MAKE IT SUITABLE FOR RECORDING TESTS ON A SERUM OF ANOTHER RH TYPE)

1 Manufacturer .... Lot No . . Date .... .

2 Agglutinin titer—Using 2 percent suspensions of the following Rh types of cells

Test cells	Greatest dilution giving a 1+ react on	
	Control serum	Serum under test
Rh ′ (CDe)		
Rh ′ (CDe)		
Rh ′ (cDE)		
Rh ″ (cDE)		
rh (Cde)		
rh (cdE)		
rh (cde)		
Average value obtained with Rh positive cells . units		

3 Has the test described in section 2 41 of the Minimum Requirements been performed?

4 Sterility test—Using not less than 0 5 ml of the product ..

5 If a dried product Moisture .. percent

(To be signed by responsible operator)

1 16¹ As stated before, if serums are available the donor's blood if Rh_0 negative should be tested with serums containing agglutinins for the rh′ factor, i e , anti Rh_0' or anti rh′ (so called 70 percent serum) serum and also for the rh″ factor, i e , anti Rh_0'' or anti rh″ (so called 30 percent serum) serums The factors rh′ and rh″, though antigenically weaker, may sensitize those lacking them

1 17 In emergencies and in the absence of serums which may detect rh′ or rh″ factors in Rh_0 negative donors, one may use the sensitive compatibility test given in section 1 17¹ to eliminate possible reactions due to rh′ or rh″ factors However, it must be kept in mind that in susceptible recipients such transfusions may sensitize them to later transfusions, and in the case of young women and girls may contribute to the later development of erythroblastosis fetalis or hemolytic disease of the newborn in fetuses bearing rh′ or rh″ factors

1 17¹ The newest methods of crossmatching avoid cell suspensions in saline and utilize whole, oxalated blood and oxalated plasma Agglutinins will be discovered that are missed by the other technics Blood from both donor and recipient is collected in dry test tubes containing an amount of dry oxalate, sufficient to prevent coagulation A portion of the sample is placed in a separate tube, centrifuged, and the supernatant plasma withdrawn The two types of mixtures are then made using, however, whole blood and oxalated plasma Probably the best method, at present, is to work with 2 percent suspensions of cells in their own oxalated plasma Make the mixtures in small test tubes such as used for Rh typing (approximately 7 mm internal diameter by 70 mm in length) Incubate in a water bath at body temperature for 1 hour, and centrifuge at 500 to 1,000 r p m for 1 minute Then gently roll the tubes to dislodge the cells from the bottom of the tubes (do not shake), and examine by naked eye and low power microscope, in the small test tube, for any agglutination

1 18 At present it would seem practicable to set requirements for Rh testing in hospital clinical laboratories and in blood banks under three headings

a The use of one anti Rh serum, i e , an anti Rh_0 serum which will identify all bloods containing or lacking the Rh_0 factor

b Testing all blood donors found Rh negative by this serum with a second serum, i e , an anti Rh′ serum or anti rh′ serum This will identify blood containing the rh′ factor alone

c Testing further all Rh negative blood donors with a third serum—anti Rh″ or anti rh″ This will identify bloods containing the rh″ factor

1 19 A good serum should be specific for the Rh factor or factors it is supposed to contain Obviously it should be completely free of agglutinins for any of the other agglutinogens found in human red

1 12 Therefore, all Rh negative individuals who will need trans fusions, especially if over a long period of time, should receive only Rh negative blood Also, all girls and all women still in the child bearing age who are Rh negative should receive only Rh negative blood There are three Rh factors which may occur in red blood cells, either alone or in combination, making the blood Rh positive These are Rh_0, Rh prime (rh′), and Rh double prime (rh″) The cells of 85 percent of the white population contain Rh , and will show aglutination with an anti Rh serum The Rh_0 factor is the strong est Rh antigen and from a practical point of view the most important An additional 1 5 percent will react with anti rh′ serum, and a fur ther 0 5 percent with anti rh″ serum

1 13 Ideally, in a transfusion service all three serums should be used to accurately identify the Rh factors in each blood (patient and donor) This is too complicated at present and must be reserved for specialists in the study of unusual cases Also, there is not enough of the three serums available for every laboratory

1 14 Therefore, Rh testing must be simplified to be made practical and to give certain minimum safeguards to all patients An analogy can be drawn with blood grouping Most transfusion accidents occur because of gross errors such as erroneous blood grouping with weak and unreliable grouping serums Similarly, gross dangers from the Rh factor must first be eliminated before one can proceed to refine ments of testing to eliminate all dangers The anti Rh serums must be available, the test must be kept simple, and must be performed with an accurate and sensitive technic

1 15 The problem is a double one In the first instance, for *re cipients* of a transfusion, it is of utmost importance to determine whether they are Rh negative or not, since Rh is the most antigenic of the Rh factors If an Rh negative individual is mistakenly called Rh positive, transfusion with Rh positive blood will likely sensitize him and subsequent transfusion with Rh blood may cause disaster All recipients, therefore, should be tested with anti Rh_0 serum (so-called 85 percent serum) Fortunately, and naturally, this is the test serum available in the largest quantity

1 151 It would be unfortunate for the blood of a *recipient* of a transfusion to be tested with anti Rh ′ (so called 87 percent serum) or anti Rh ″ (so called 85 5 percent serum) serum alone, since these serums may give reactions in patients whose cells are Rh_0 negative yet contain rh′ or rh factors Transfusion of Rh positive blood will sensitize individuals for the rh′ or rh type to the Rh factor, and thus they should receive only Rh_0 negative blood

1 16 Secondly, it is most important that the blood of all *donors* be tested with anti Rh serum Rh_0 cells should never be given to Rh negative individuals

1 2 4 In both of these tests blocking antibodies do not interfere with agglutination as they do with the usual tests with saline suspensions of cells In fact, in the presence of blocking antibodies, agglutination is increased Both of these tests are proving of great value in detecting weak agglutinins and blocking antibodies in individuals sensitized to the Rh factors The progress of sensitization can be followed in pregnant women with a history of bearing infants with congenital hemolytic disease This will serve as a guide to the obstetrician and to preparations for transfusing the baby when it is born

2 TECHNICAL CONSIDERATIONS INVOLVED IN ANTI RH TYPING SERUMS

2 1 *Rh blood types*—The determination of Rh types of human red blood cells depends upon the use of specific and potent anti Rh serums In the beginning it will be necessary to obtain such serums from a reliable source The following system may be followed in order to determine the Rh blood types.

2 1 1 Agglutination reactions are set up using 2 percent suspensions of cells in test tubes with three anti Rh serums, anti Rh_0, anti rh′, and anti rh″ The results of the reactions of five anti Rh serums with cells of the eight different Rh types of blood are tabulated below

Rh blood type	Reaction with anti Rh serums[1]				
	Anti Rh 85 percent	Anti rh 70 percent	Anti rh″ 30 percent	Anti Rh 87 percent	Anti Rh 85.5 percent
rh (Rh negative)	−	−	−	−	−
Rh_0	+	−	−	+	+
Rh	+	+	−	+	+
Rh	+	−	+	+	+
Rh Rh	+	+	+	+	+
rh	−	+	−	+	−
rh	−	−	+	−	+
rh rh	−	+	+	+	+

[1] Percentages shown are approximate percent of bloods of mixed white (U S) population agglutinated by serum

By this method laboratories concerned with the production of anti Rh serum should determine the Rh blood types of readily available donors for cells to be used in titrating anti Rh serums

2 2 *The degree of specificity of an Anti Rh typing serum*—Certain anti Rh (anti D) serums intended for use by the slide test method appear to contain weak and slower acting antibodies for rh′ (Cde) cells The clumping of rh′ cells with these serums may be noted also in tube tests using serum or albumin

blood cells, such as A or B substances If the human serum contains anti A or anti B agglutinins, these must be removed by absorption with group A_1 or group B red blood cells, or by neutralization with solutions of A and B substances, or by a combination of both methods.

1 2 If anti Rh serum is produced in animals, similarly, any naturally occurring, or induced, anti A, anti B, or anti O agglutinins must be removed It must be remembered that the serum of most domestic animals contains some or all of these agglutinins which are both avid and high titered, especially in the presence of a sufficient amount of human serum or plasma or a solution of human or bovine albumin

1 21 The test tube test for Rh factors is performed as follows Place in a small test tube with an inside diameter of 7 mm one drop of a 2 percent suspension of once washed red blood cells in 0 9 percent saline, and one drop of anti Rh serum An additional drop of 0 9 percent saline may be added The tubes are placed in a water bath at body temperature for 1 hour and centrifuged for 1 minute at 500 to 1,000 r p m The tube then is gently rotated to dislodge the cells from the bottom and to observe if agglutinates are present If they are not easily noticed macroscopically, then the tube is placed on its side and the contents looked at through a very low power of the microscope to determine if microscopic agglutination is present. Microscopic agglutination signifies a positive reading as much as macroscopic Care must be taken not to shake or tap the tube too roughly because Rh agglutination is easily broken up, and a positive test would be read as a negative one

1 22 Another widely used test is a slide test described by Diamond and Abelson In this a large drop of oxalated blood is mixed on a slide with a small drop of specially selected anti Rh serum (Blood or cells suspended in physiological saline cannot be used in this test) The slide is placed on the warmed ground glass top of an electrically lighted illuminating box, which is hinged and can be tilted forward and backward Rh positive bloods show easily observed macroscopic agglutination usually in less than 3 minutes This test has proved to be reliable, does not fail to identify Rh positive bloods, and does not give false positives It is of value, also, when Rh determinations are needed in a hurry as it can be completed quickly after a small amount of oxalated blood is obtained

1 23 Wiener has devised a test which he has called a "conglutination" test. This is carried out in small tubes, as is the usual Rh test A drop of a 2 percent suspension of blood or cells in homologous serum or oxalated plasma, or in group AB serum, is placed in the tube and a drop of anti Rh serum added Incubation and reading are as usual Heavier suspensions of blood may be used This is a reliable and very sensitive test.

1 24 In both of these tests blocking antibodies do not interfere with agglutination as they do with the usual tests with saline suspensions of cells In fact, in the presence of blocking antibodies, agglutination is increased Both of these tests are proving of great value in detecting weak agglutinins and blocking antibodies in individuals sensitized to the Rh factors The progress of sensitization can be followed in pregnant women with a history of bearing infants with congenital hemolytic disease This will serve as a guide to the obstetrician and to preparations for transfusing the baby when it is born

2 TECHNICAL CONSIDERATIONS INVOLVED IN ANTI RH TYPING SERUMS

2.1 *Rh blood types* —The determination of Rh types of human red blood cells depends upon the use of specific and potent anti Rh serums In the beginning it will be necessary to obtain such serums from a reliable source The following system may be followed in order to determine the Rh blood types.

2 11 Agglutination reactions are set up using 2 percent suspensions of cells in test tubes with three anti Rh serums, anti Rh , anti rh', and anti rh ' The results of the reactions of five anti Rh serums with cells of the eight different Rh types of blood are tabulated below

Rh blood type	Reaction with anti Rh serums[1]				
	Anti Rh 85 percent	Anti rh [illegible]0 percent	Anti rh'' 30 percent	Anti Rh 8[illegible] percent	Anti Rh '' 65.5 percent
rh (Rh negative)	−	−	−	−	−
Rh_o	+	−	−	+	+
Rh	+	+	−	+	+
Rh ''	+	−	+	+	+
Rh ' Rh	+	+	+	+	+
rh	−	+	−	+	−
rh	−	−	+	−	+
rh rh	−	+	+	+	+

[1] Percentages shown are approximate percent of bloods of mixed white (U S) population agglutinated by serum

By this method laboratories concerned with the production of anti Rh serum should determine the Rh blood types of readily available donors for cells to be used in titrating anti Rh serums

2 2 *The degree of specificity of an Anti Rh typing serum* —Certain anti Rh_o (anti D) serums intended for use by the slide test method appear to contain weak and slower acting antibodies for rh (Cde) cells The clumping of rh' cells with these serums may be noted also in tube tests using serum or albumin

34[illegible] —[illegible]——6

2 21 The difficulty in removing the weaker antibody is recognized However, serum intended for use as an anti Rh_o slide testing serum should give reactions only with cells containing Rh_o (D) factor within the time limits and conditions recommended in the directions for use of the serum If such serum reacts with rh′ cells after the prescribed time (or, for example, in tube tests with more dilute cell suspensions) a detailed explanation should be provided the user and methods recommended to prove the specificity of the cell in question

2 22 At this time it appears impractical to discard potent serums which give excellent results by the method of testing advised but which may lack specificity if the accompanying directions are not followed However, the goal of providing completely specific diagnostic serums is clear, and every effort should be made to produce such serums Selection of cell donors and recipients who are to provide specific serums would appear to be of great importance Meanwhile, the usefulness of the serum containing traces of antibodies other than those appearing on the label should be judged on the chance with which errors are apt to be made by technicians of average, or less, ability—obviously errors should not occur

2 23 The same limitations of usage apply to serums containing antibodies of the blocking variety whose labeled specificity is based on the action of the serum on test cells suspended in isotonic saline Since definite techniques must be followed in their use, the direction circular should be explicit both as to technique and the possible variant results which might be obtained by deviating from the recommended technique

2 24 In addition to tests of anti Rh serum of the blocking variety with Rh′, Rh_o'', and rh cells, it is recommended that tests with rh′ (and rh″, if available) cells be included on the release protocols

3 Undesirable Qualities in Anti Rh Typing Serums

3 1 *Autoagglutination*—Serum to be used in blood grouping tests should be free of so called autoagglutinins As a rule, autoagglutination is apparent when serum and red cells from the same individual are placed at temperatures lower than room temperature (preferably 2° to 6° C), agglutination is weakened or abolished at 37° C Occasionally serums are found which agglutinate autologous cells at room temperature These reactions are based on the presence of an absorbable agglutinin in the serum and an agglutinogen in human red cells It acts on all human red cells irrespective of group or type Obviously, serum containing autoagglutinins which are active at room temperatures cannot be used as blood grouping or anti Rh typing serum

3 2 *Bacteriogenic agglutination*—It is well known that red cell suspensions after being contaminated by the growth of certain bacteria

may become agglutinable by serum, regardless of the group or type of cells This phenomenon may be avoided by the use of fresh blood cell suspensions

3 21 Contamination of serum by the growth of certain bacteria may also lead to false positive agglutination reactions Careful, sterile technic in entering serum containers and the presence of an effective preservative will prevent this undesirable reaction.

[Federal Security Agency
National Institutes of Health
Bethesda 14, Md.
6th revision, January 15, 1951]

V. Minimum Requirements: Normal Human Plasma

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1 The Product

1 1 *Proper name*—The proper name of this product is Normal Human Plasma

1 2 *Source*—Determination of the suitability of the donor shall be the responsibility of a licensed physician and shall be done by him or under his supervision with the assistance of the necessary trained attendants Only those persons may serve as a source of normal human plasma who are in physical condition to give blood, whose temperature is normal and whose blood pressure is such that blood donation will not be harmful to the donor Donors shall be free of disease transmissible by transfusion of plasma, as far as can be determined from the personal history and from such physical examination and clinical tests as appear necessary for each donor on the day the blood is obtained Serological tests for syphilis need not be done if the blood is to be used only for the preparation of normal human plasma As an added precaution a history of viral hepatitis shall be cause for rejecting the donor Existing pregnancy or pregnancy within the preceding 6 months shall disqualify a donor[1]

1 2 1 Only persons having not less than 12 5 gm of hemoglobin per 100 ml of blood should serve as donors If the copper sulfate specific gravity method is used to determine hemoglobin, a specific gravity of not less than 1 053 shall be used as indicating adequate hemoglobin[2]

1 3 *Protection of donor*—The preparation of areas of the skin used for the collection of blood or for injections incidental to blood collection shall be adequate to protect the donor against infection Apparatus or instruments capable of transmitting infection to the donor, such as bloodletting devices, needles and syringes, shall be sterilized prior to use for each donor Such instruments must be sterilized by heat by boiling in water for 20 minutes or by autoclaving

2 Collection of the Plasma

2 1 *Method of bleeding the donor*—The method employed for the removal of blood from the donor shall conform to the accepted standards of aseptic surgery and shall be made in a closed system as defined in section 2 5

2 1 1 *The bleeding clinic*—Drawing blood from the donor shall be the responsibility of a licensed physician and shall be done by him or under his supervision with the assistance of the necessary trained attendants The drawing may be performed in a suitable

[1] Amendment of August 20 1952

[2] *Measurements of Specific Gravity of Whole Blood and Plasma by Copper Sulfate Solutions* Robert A Phillips Donald D Van Slyke Paul V Hamilton Vincent P Dole Kendall Emerson Jr and Reginald M Archibald J B C 183 No 1 305-360 1950

bleeding room located in the licensed laboratory, or some other place having suitable space and equipment. Irrespective of the place of bleeding, the personnel, the space, and the equipment must be the responsibility of the establishment under whose license the blood is drawn.[8]

2.2. *The anticoagulant solution*.—The apparatus used for the removal of the blood and the receiving unit shall be chemically clean and sterile. *The receiving unit shall contain an anticoagulant solution* of suitable composition. If the blood is to be processed without delay to either liquid or dried plasma, a pyrogen free anticoagulant solution of the following composition shall be used:

Tri sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	4.00 gm.
Water for injection (USP) to make	100 ml.

Add 10 ml. of this solution to the receiving unit before its sterilization for each 100 ml. of blood to be drawn.

2.2.1. If the blood is to be used as whole blood or if the red blood cells are to be used as resuspended (or packed) red cells, in addition to processing the plasma as liquid plasma, one of the acid citrate dextrose (ACD) anticoagulant solutions described in the Minimum Requirements for Citrated Whole Blood must be used.

2.3. *The bleeding clinic*.—Drawing blood from the donor shall be performed under the general supervision of a qualified doctor of medicine with the assistance of the necessary trained attendants.

2.3.1. The drawing of blood may be performed in a suitable bleeding room under the control of the processing laboratory or blood may be supplied by a licensed laboratory to another licensed laboratory for processing. Blood so supplied shall be adequately identified as to name or number of donor, date of bleeding, and name and license number of bleeding establishment.

2.4. *Separation of the plasma*.—Each bleeding shall be drawn into its own receiving bottle, which shall be the same container used later for centrifuging the individual bleeding and for separating the plasma from the red cells. Pooling of whole blood before separating the plasma is not permitted. Within 1 hour after bleeding the blood shall be placed in a cold chamber which shall have a temperature range of 2° to 10° C. (Freezing must be avoided.) If transportation of the blood before removal of the red cells, from bleeding clinic to processing laboratory, becomes necessary, it shall be transported in shipping cases provided with refrigeration sufficient so that (*a*) if the blood has already been cooled as specified above, the temperature of each individual blood will not be in excess of 10° C. on arrival at the processing laboratory, or (*b*) if the blood is shipped before its temperature has been reduced to 2° to 10° C., the refrigeration

[8] Amendment of August 20, 1952.

capacity of the shipping case shall be such as to insure continued reduction of the temperature of each blood during the transportation interval

2 41 Immediately upon arrival at the processing laboratory the blood shall be placed in a cold chamber having a temperature range of 2° to 10° C (preferably 1° to 6° C) The blood may be removed from the cold chamber during the interval required for freeing the plasma of cells It is recommended that a cooled centrifuge be used for the removal of the red cells Following centrifugation and pooling, distribution into the final containers and shell freezing shall follow without needless delay in order to prevent temperature fluctuations of the plasma which tend to accelerate fibrin precipitation When processing liquid plasma it may be allowed to reach the recommended storage temperature (see 9 1) as soon as desired after separation from the cells

2 42 If the whole blood has been drawn into sodium citrate anticoagulant solution, the blood shall be centrifuged within 72 hours of the time of drawing the blood from the donor, and the plasma shall be separated from the cells within 96 hours of the time of drawing the blood from the donor

2 43 However, if an ACD solution has been used as the anticoagulant and red cell preserving solution, the plasma may be separated from the cells by centrifugation, or by sedimentation, at any time not later than 5 days after the expiration date of the whole blood

2 5 *The closed system defined*—All transfers of blood from patient to the receiving bottle and of plasma from one container to another shall be made in a closed system A closed system is a system which permits the transfer of material from one container to another entirely within the system without contamination through exposure to external conditions It is accomplished either through an exchange of position of the materials within the system or through the introduction of sterile air as transfer is being made by the application of negative or positive pressure All air for replacement must first pass through a suitable bacteria excluding filter, except that when the transfer is being made in a closed "sterility room" equipped with mechanical or physical air sterilizing devices of proven quality, and in operation when the transfer is being made, the air for replacement need not be passed through such a filter

3 Preparation of the Plasma Pool

3 1 *The number of donors per pool*—The supernatant plasma shall be drawn from the bleeding bottle after adequate centrifugation or sedimentation through a closed system into the pool bottle Only pooled human plasma shall be placed in the final container and for

this purpose a minimum of eight individual plasmas shall constitute a pool

3 2 *Description of the pooling apparatus*—It is recommended that the end of the closed system apparatus which is introduced into the plasma be fitted with a glass or metal tube of suitable caliber and that the shaft of this tube be enclosed in a suitable flexible sheath of rubber or other equivalent material The upper end of the sheath is attached to the rubber tubing where it joins with the above mentioned glass or metal tube and the lower end is fitted around the neck of a glass bell, the dimensions of which are such that the bell will not touch the lip of the bleeding bottle when the rim is resting on the shoulder of the bleeding bottle When the protective sheath is fully extended, the above glass or metal tube should reach through the neck of the glass bell and slightly beyond In addition it is recommended that the rim of the glass bell be fitted with a rubber washer designed to fit closely to the shoulder of the bottle and also that the bell be fitted with a cotton stopper side arm suitable as a sterile air intake opening When in operation the glass bell is placed over the mouth of the plasma containing bottle so as to rest on the shoulder of this bottle in order that the above glass or metal tube may be inserted into the plasma The exposed end of the glass or metal tube should be protected by a suitable device so that the plasma will be drawn into the tube from the sides rather than from below

3 3 *Clarification of the plasma*—Pooled plasma should be clarified to remove particulate matter which may interfere with the sterilization by ultraviolet light and to improve the physical appearance of the finished product, particularly when processing to liquid plasma

3 4 *Sterilization by ultraviolet light*—Pooled plasma shall be sterilized by ultraviolet light in accordance with the Minimum Requirements for Ultraviolet Irradiation for Sterilization of Biologic Products

3 5 *Liquid plasma to contain dextrose*—If the plasma is to be prepared as liquid plasma, it shall be stabilized by the addition of dextrose to give a concentration of 5 percent dextrose USP in the final product Dextrose shall not be added to plasma which is to be distributed as frozen or dried plasma

4 Tests for Sterility

4 1 *Sterility test on pooled plasma before sterilization*—A total bacterial count by dilution shall be made in fluid thioglycollate medium on each pool of plasma on a sample withdrawn from the completed pool immediately before sterilization by ultraviolet light A pool having an initial total bacterial count of not more than 10 bacteria per ml is acceptable after ultraviolet irradiation provided it meets the final container sterility test (see 4 3, 4 4, 4 5) A pool

having an initial total bacterial count in excess of 10 bacteria per ml is acceptable after ultraviolet exposure only provided it meets the final container sterility test and in addition the test for pyrogens (sec 5 4)

4 11 The sterility test is made by culturing suitable dilutions in fluid thioglycollate medium (sec 4 2) Incubation is for not less than 7 days at 32° C

4 2 *Culture medium for the sterility test*—The approved culture medium for the sterility test is fluid thioglycollate medium described in detail in the National Institute of Health Circular Culture Media for the Sterility Test Because of the viscosity of the fluid thioglycollate medium it is essential that the inoculum be thoroughly mixed into the medium It is also important that the same ratio of surface exposed to volume of medium in culture tube be observed as is described in section 6 of the circular (see appendix C for the formula)

4 3 *Sterility test on the final container of liquid plasma*—When liquid plasma is being processed, a sterility test shall be made on plasma from at least one final container This test shall be made at least 2 weeks after filling During this interval all containers shall have been stored at a uniform temperature within the range 15° to 30° C as required in section 9

4 31 At the end of the storage period of at least 2 weeks, visually inspect each final container in the lot, using a transmitted light of suitable intensity Discard all containers showing any unusual sediment or clouding From the remaining containers select one at random for the sterility test (sec 4.32) Repeat the visual inspection after the completion of the sterility test, discarding all containers showing any unusual sediment or clouding A lot is not satisfactory for distribution if more than 5 percent of the containers have been discarded in the two inspections

4 32 Not less than 10 ml per liter of irradiated plasma for pools up to 50 liters total volume shall be cultured for sterility For each liter over 50 liters in the pool, an additional 5 ml of plasma per liter shall be cultured for sterility The culture is made in one or more containers of culture medium and incubation is at 32° C for not less than 7 days If contamination appears, the test may be repeated as a check on technique The entire lot shall be discarded or resterilized if the presence of a contaminant is confirmed If a lot is resterilized, it must pass the sterility and inspection tests (sec 4 3) and the pyrogen test (sec 5 4)

4 4 *Sterility test on the final container of frozen plasma*—For this test a final container shall be selected at random from the final containers frozen as directed in section 6.3 and stored as directed in section 9 41 The container for the sterility test is then thawed as directed in section 6 3

4 41 The volume of the test sample and the performance of the sterility test is the same as is required of liquid plasma (sec 4 32) Any unused portion of the sample, if otherwise satisfactory, may be returned to a subsequent plasma pool before its sterilization or may be used in plasma fractionation

4 5 *Sterility test on the final container of dried plasma*—The plasma sample for the sterility test shall be taken from a final container selected at random and subjected to shell freezing and drying under operating conditions similar to those used in processing the rest of the lot It is restored to its original liquid volume by the addition of sterile diluent The quantity tested shall be 5 ml per liter for pools up to 50 liters total volume and an additional 2 5 ml for each liter over 50 liters in the pool

4 51 The performance of the sterility test is the same as is required of liquid plasma (sec 4 32) Any unused portion of the sample, if otherwise satisfactory, may be returned to a subsequent plasma pool before its sterilization or may be used in plasma fractionation

5 Other Tests

5 1 *Potency test*—Normal human plasma does not lend itself to a potency test However, the total protein of pooled plasma falls within the total protein range of plasma obtained from normal adult blood, after adjustment has been made for the volume of anticoagulant solution or other diluent present

5 2 *Identity test*—The collection and processing of human plasma is conducted in laboratory space set aside for that purpose so that the accidental introduction of plasma or serum of lower animals is an impossibility Under these circumstances a specific identity test need not be performed

5 3 *Safety test*—A safety test other than the required sterility tests, and when indicated a pyrogen test, is not required

5 4 *Pyrogen test*—A pyrogen test, if required by section 4 1, is made on final container material This test is performed as described in appendix A, except that the test is acceptable if the temperature rise is less than 1 1° C (2 0° F) on each of three hourly readings in each of three rabbits following the intravenous injection of 3 0 ml per kilo of rabbit weight

5 5 *Moisture content of dried plasma*—The final dried product shall contain not more than 1 00 percent of moisture at any time during the dating period (sec 9 3) "Moisture" is defined as that weight which is lost when the dried plasma is exposed over P_2O_5 in a vacuum chamber (See appendix D for the method of determining moisture)

5 6 *Hemoglobin content*—Plasma from an individual bleeding shall be pooled only if the hemoglobin content does not exceed 25

mg per 100 ml as estimated by gross examination The completed pooled plasma shall contain not more than 25 mg of hemoglobin per 100 ml (See appendix B for a method of determining hemoglobin)

6 THE FINAL CONTAINER

6 1 *Type to be used*—The final container shall be of USP Type I, II or IV glass closed after filling with an air, moisture, and bacteria impermeable seal and shall be constructed to withstand all ordinary handling and shipping hazards without danger of breaking or otherwise impairing the product The container shall be equipped so that the necessary connections for the injection of the plasma into the recipient may be attached easily, directly, and aseptically to the closure of the plasma container

6.2 *Method of filling*—The final containers are filled through a closed system by applying sterile air pressure in the pool bottle The entire pool is filled into final containers during a single filling operation A filter, adequate for the removal of particulate matter which is potentially dangerous in intravenous therapy, shall be placed in the filling system between the pool bottle and the final container

6 21 Filling the final containers shall begin promptly after completing the pool and without waiting for the report of the sterility test The elapsed time from donor to final container is detailed in section 2 4 but the objective at all times should be to bring the plasma to its final state in the final containers as quickly as good operating technique will permit

6 3 *Method of handling plasma to be frozen*—Plasma to be processed to the frozen state shall be frozen as soon as possible after filling by a method that will freeze all of the plasma within a period of 6 hours Each bottle of frozen plasma should be accompanied by instructions for melting prior to use For melting the frozen plasma should be placed in a constant temperature device maintained at a temperature not above 37° C The melted plasma should be mixed in the bottle and administered within a period of 3 hours after melting is completed

6 4 *Method of handling plasma to be dried*—When plasma is to be processed to the dried state, shell freezing shall follow filling without needless delay and the drying is preferably begun without delay after shell freezing However, the shell frozen plasma may be stored at a temperature lower than minus 18° C to await the outcome of the sterility test or to await a more convenient time for carrying out the drying process In either event, the final disposition of the entire lot shall depend upon the results of the test for sterility (sec 4 5) and for moisture content (sec 5 5) Drying shall be accomplished by a method which is not deleterious to the plasma constituents and which will result in a readily soluble and sterile product

7 The Diluent for Dried Plasma

7 1 *The container* —A suitable container of USP Type I, II, or IV glass holding the necessary amount of pyrogen free, sterile, and otherwise suitable diluent shall accompany each container of dried plasma This container shall be closed after filling with an air, moisture, and bacteria impermeable seal

7 11 The quantity of diluent in the container shall be at least sufficient to restore the dried plasma to the volume of original plasma The container shall be equipped so that the necessary connections for the transfer of the diluent to the dried plasma may be attached easily, directly, and aseptically

7 2 *The diluent* —The diluent is 0 1 percent solution of citric acid in pyrogen free sterile distilled water The proper name for this diluent is Water for Injection, but the label shall carry the additional designation "Sterile, Pyrogen free, containing 0 1 percent Citric Acid" Each lot of diluent shall pass satisfactory tests for sterility and pyrogenicity prior to release (See appendix A, pyrogen test)

8 Accessory Equipment

8 1 Accessory equipment such as needles, tubing, and airway furnished with the final container of plasma shall be sterile and pyrogen free

9 General Requirements

9 1 *Preservative* —No preservative is added Reliance is placed entirely on good blood collection and processing techniques followed by prompt sterilization by ultraviolet radiation The label on the final container shall bear the following statement "No preservative added but treated with ultraviolet radiation"

9 2 *Labeling* —The labels shall meet the labeling requirements as stated in the Regulations In addition the following must be included (1) the volume of pooled original plasma represented, (2) the statement as to the method of sterilization specified in section 9 1, (3) in the case of liquid or frozen plasma a statement indicating the actual volume, (4) in the case of liquid plasma the amount of added dextrose and (5) in the case of dried plasma on the outside label the warning 'Do Not Freeze' The statement "No U S Standard of Potency' need not appear

9 21 The filling volume for liquid, frozen, or dried plasma shall be equal to the stated volume of original plasma represented plus the volume of anticoagulant solution or other liquid added to the blood or to the plasma

9 22 It is recommended that either the label or an accompanying circular indicate that (1) plasma should not be warmed before administration, (2) that a suitable filter must be placed in the lumen

of the tube leading to the recipient and (3) if dried plasma, full directions for dissolving and the manner of administration.

9.23 It is recommended that either the label or an *accompanying* circular for dried plasma carry the statement "Use within 3 hours after restoration."

9.24 Either the label or circular for normal human plasma must carry a warning statement to the effect that despite careful donor selection and ultraviolet irradiation, the plasma may contain the virus of homologous serum hepatitis.**

9.3 *Dating.*—The expiration date of liquid plasma is 2 years and of frozen plasma 5 years after the date of manufacture. The expiration date of dried plasma is 5 years after the date of manufacture or date of issue provided the date of issue is not more than 1 year after the date of manufacture. The date of manufacture is the date of bleeding the donor.

9.4 *Storage.*—Liquid plasma shall be stored continuously as nearly as possible at a uniform temperature within the range 15° to 30° C.

9.41 Frozen plasma shall be stored continuously at minus 18° C or lower.

9.42 Dried plasma may be stored at prevailing temperature not exceeding 37° C (98.6° F.) provided freezing of the final package containing diluent is avoided. The preferred temperature is 2° to 10° C.

9.5 *Requirements for release.*—Plasma may be released by the manufacturer when the necessary tests have been completed for each lot. A sample, accompanied by a protocol detailing the information indicated in appendix L shall be submitted to the National Institutes of Health, Laboratory of Biologics Control, when specifically requested. The manufacturer shall not be required to keep reference samples from each lot. (See sec. 22.36 (e) of the Regulations.)

** Amendment of February 3, 1953.

complete. Subsequent dilution is made in plasma which is free of all visible trace of hemoglobin color. The hemoglobin content of the blood used for reference in this comparison is determined by a method having an accuracy equal to that obtainable by the use of a Sahli apparatus equipped with permanent color standards which have been calibrated against the Van Slyke oxygen capacity method, Wong iron method, or their recognized equivalents. (Amer. J. Clin. Path., 3: 85-95, 1933 and 4: 354-361, 1934.)

APPENDIX C

Fluid Thioglycollate Medium for the Sterility Test

Full details of the formula and method of preparing the fluid thioglycollate medium are given in the National Institutes of Health Circular "Culture Media for the Sterility Test." For convenience sections 3, 4, 5, and 6 of that circular are included in this appendix. The complete fluid thioglycollate medium in the dehydrated form may be purchased from at least two manufacturers.

3. Fluid Thioglycollate Medium

Ingredient	Amount
l-Cystine (Reagent)	0.75 gm.
Sodium chloride	2.5 gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	5.5 gm.
Granular agar (Less than 15-percent moisture by weight)	0.75 gm.
Water soluble extract of yeast	5.0 gm.
Pancreatic digest of casein	15.0 gm.
Distilled water	1,000.0 ml.
Sodium thioglycollate	0.5 gm.
or	
(Thioglycollic acid	0.3 ml.)
0.10 percent solution of Resazurin (Freshly prepared)	1.0 ml.

Some difficulty may be experienced in getting the l-cystine into solution. (Hydrochloric acid may not be used as an aid in dissolving.) One method is to mix in a mortar all of the dry ingredients except the sodium thioglycollate (or the thioglycollic acid) in the order given in the table, thoroughly mixing each as it is added. Then stir in a portion of the water (previously heated), transfer to a suitable container, add the remainder of the water and complete the solution by heating in a boiling water or steam bath. Then add the sodium thioglycollate or thioglycollic acid. Irrespective of the method used,

it is preferable to add the sodium thioglycollate or thioglycollic acid after the preliminary heating Adjust the reaction with sodium hydroxide to such a point as experience shows will result in a pH of 7 1—0 1 in the completed and sterilized medium Reheat, but do not boil, and filter (only if needed for clarification) through a moistened paper filter, then add the Resazurin solution Distribute into final containers and sterilize in the autoclave for 18-20 minutes at 121° C to 123° C

4 *Storage of the medium* —After removal of the final container of medium from the autoclave, cool promptly to 25° C in order to set the agar Store at 15° C to 30° C (preferably 20° C to 30° C as low temperature increases absorption of oxygen from the atmosphere), avoid excessive light If more than 30 percent of the uppermost portion of the medium has changed to a pinkish color, it is unsuitable for use Under such circumstances one reheating in a boiling water or steam bath is permissible in order to drive off the absorbed oxygen

5 *Growth promoting quality of medium* —It is recommended that each lot of medium be tested for its growth promoting and oxidation reduction qualities For this purpose use one or more bacteria that are exacting in growth requirements At the end of the incubation period used for the sterility test (7 days) less than 60 percent of the medium in each tube shall have changed color

6 *Type of container for culturing* —This medium permits the growth of both aerobic and anaerobic organisms in the same open container The test tube of choice is one which measures 20 x 150 mm into which is placed 15 ml of culture medium This will provide adequate medium for inoculum up to 3 ml and adequate thioglycollate to inactivate a mercurial preservative when present in the inoculum in not more than a 1 10,000 dilution Other preservatives will need to be inactivated by adequate dilution unless an effective inactivator is available and used With a large volume of inoculum, cylindrical or square bottles having approximately the same ratio of surface exposed to volume of medium as mentioned above are recommended The inoculum must be mixed thoroughly into the medium in all instances because of the viscosity of the medium Likewise, the contents of the inoculated tubes should be remixed at the time of making the first, or 48 hour reading in order to distribute the growth more widely through the medium This will insure a more accurate final reading

817090 —50——7

complete. Subsequent dilution is made in plasma which is free of all visible trace of hemoglobin color. The hemoglobin content of the blood used for reference in this comparison is determined by a method having an accuracy equal to that obtainable by the use of a Sahli apparatus equipped with permanent color standards which have been calibrated against the Van Slyke oxygen capacity method, Wong iron method, or their recognized equivalents. (Amer. J. Clin. Path., 3: 85–95, 1933 and 4: 354–361, 1934.)

APPENDIX C

Fluid Thioglycollate Medium for the Sterility Test

Full details of the formula and method of preparing the fluid thioglycollate medium are given in the National Institutes of Health Circular "Culture Media for the Sterility Test." For convenience sections 3, 4, 5, and 6 of that circular are included in this appendix. The complete fluid thioglycollate medium in the dehydrated form may be purchased from at least two manufacturers.

Table 3. Fluid Thioglycollate Medium

Ingredient	Amount
l-Cystine (Reagent)	0.75 gm
Sodium chloride	2.5 gm
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	5.5 gm
Granular agar (Less than 15 percent moisture by weight)	0.75 gm
Water-soluble extract of yeast	5.0 gm
Pancreatic digest of casein	15.0 gm
Distilled water	1,000.0 ml
Sodium thioglycollate	0.5 gm
or	
(Thioglycollic acid	0.3 ml.)
0.10-percent solution of Resazurin (Freshly prepared)	1.0 ml.

Some difficulty may be experienced in getting the l-cystine into solution. (Hydrochloric acid may not be used as an aid in dissolving.) *One method is to mix in a mortar all of the dry ingredients* except the sodium thioglycollate (or the thioglycollic acid) in the order given in the table, thoroughly mixing each as it is added. Then stir in a portion of the water (previously heated), transfer to a suitable container, add the remainder of the water and complete the solution by heating in a boiling water or steam bath. Then add the sodium thioglycollate or thioglycollic acid. Irrespective of the method used,

APPENDIX E

RELEASE PROTOCOL FOR NORMAL BLOOD PLASMA

1 MANUFACTURER	DATE	LOT NO

2 POOLING DATA

a Date of drawing oldest blood

Date of pooling

b Anticoagulant in blood

c Records of donors available in processing laboratory?

☐ Yes ☐ No

d Number of donors contributing to pool

e Method of clarification preliminary to irradiation

f Total Volume ____ liters

g Bacterial count before irradiation ____ organisms per ml

3. IRRADIATION DATA

a Ultraviolet lamp intensity measured prior to use?

☐ Yes ☐ No

b Time required for irradiation ____ hrs ____ min

c Irradiation Control

No A aerogenes/ml in control sample

Before irradiation ____

After irradiation ____

Age of culture used for control test ____ hrs

4 OTHER PROCESSING DATA

a Filtration with bacterial filter? ☐ Yes ☐ No

b Inspected for particulate matter? ☐ Yes ☐ No

c If distributed as dried plasma

c' If distributed as liquid plasma

Dextrose concentration ____ %

Analyzed? ☐ Yes ☐ No

First inspection of filled product for unusual sediment or clouding

APPENDIX D

A Method for the Determination of Residual Moisture

The amount of residual moisture remaining in a product which is labeled as dried shall contain not more than 1 00 percent moisture when determined by the following method "Expose a 1 to 2 gm sample of the product accurately weighed to the third decimal, evenly distributed in a weighing bottle not less than 60 mm in diameter, in a vacuum desiccator at less than 1 mm pressure, over fresh phosphorus pentoxid, and at room temperature until the weight remains constant to the third decimal " In removing the sample to the weighing bottle, it is important to avoid unnecessary exposure of the dried product to the air, particularly if the moisture content of the atmosphere is relatively high Samples of less weight may be used in smaller weighing bottles for the determination of moisture in products where full samples are not available

SELECTED CIVIL DEFENSE PURCHASE DESCRIPTIONS, BASED ON SPECIFICATIONS OF THE ARMED FORCES MEDICAL PROCUREMENT AGENCY

Contents

Nomenclature	Page
Bottle vacuum blood with anticoagulant, 600 cc, 6 s	98
Blood donor set, indirect blood transfusion	99
Blood recipient set, indirect blood transfusion	100
Blood grouping serum, anti A dried 75 test	101
Blood grouping serum anti B, dried 75 test	103
Blood typing serum anti Rh dried equivalent to 3 cc	105
Blood grouping serum anti A and anti B (group O) dried 75 test	107
Albumin serum bovine 30 percent 20 cc	109
Cupric sulfate 170 gm special reagent for measuring specific gravity for blood	110
Blade operating knife No 11 6 s	111
Container blood shipping	113
Plasma, normal human dried irradiated donated 500 cc	114
Plasma, normal human dried irradiated commercial 500 cc	115
Dextran injection 6 percent 500 cc	117
Polyvinyl pyrrolidone injection M-1 3 5% 500 cc	120
Bottle vacuum blood with dextrose 2 000 cc 6 s	123
Bottle vacuum blood 500 cc 6 s	125
Blood aspirating donor set	126
Needle aspirating 13 gauge 10 and 15 inch	127
Syringe luer 2 cc and 50 cc	128
Thioglycollate fluid medium 1 lb	129

Time from pooling to frozen state ______ hrs

Temperature of storage preliminary to drying ______ °C

Time for drying

Moisture in this lot or Desiccator load ______ Maximum %

Time for reconstitution ______ seconds ☐ Not done

c' If distributed as frozen plasma

Time from pooling to frozen state ______ hr

Temperature of storage ______ °C

Inspected after ______ days storage at ______ °C

Number of containers in lot

Number discarded

Final inspection of filled product for unusual sediment or clouding

Inspected after ______ days storage at ______ °C

Number discarded

Discarded containers contaminated?

☐ Yes ☐ No ☐ Not tested

d Has the lot passed satisfactory test for

Sterility

Identity

Pyrogens

(Attach protocol of pyrogen test if test was made)

5 RECIPIENT SET (If included in package as marketed)

a Does recipient set have identifying number? ☐ Yes ☐ No

b Have these recipient sets passed satisfactory test for

Sterility? ______ Pyrogen? ______

6 RECONSTITUTING SOLUTION for this lot of plasma

Lot No (s)

Are tests satisfactory for

Sterility? ______ Pyrogens? ______

REMARKS

Item No CD V-155

Reference Military Specification MIL-B-16565 (BuMed) Military Medical Purchase Description (latest revision)

Federal Civil Defense Administration

Purchase Description

Stock Number	3-103-565 Unit Set
Nomenclature	Blood donor set, indirect blood transfusion
Description	The donor set shall be suitable for drawing of blood and transfer to the donor bottle, it should consist of one 15 gage, 1 inch hose hub needle enclosed in a sleeve to maintain sterility, one 17-gage, 1½-inch intravenous type donor needle enclosed in a sleeve to maintain sterility, one 24 inch length transparent vinyl plastic tubing, one set of instructions, all in accordance with applicable paragraphs of Military Specification MIL-B-16565
Material	Materials in the components of the donor set shall be in strict accordance with Military Specification MIL-B-16565
Sterility	The fluid path of the donor sets and the needles shall comply with the requirements of the United States Pharmacopoeia sterility tests for solids
Packaging, Packing, Marking	Each complete donor set shall be packaged in a unit container, of suitable size and design so constructed with interior fittings to adequately protect the contents and prevent formation of elbows in the tubing Unless otherwise specified, 288 donor sets shall be packed with suitable intermediate packing in an exterior container of suitable size and design so constructed to insure safe delivery to destination Marking of interior and exterior containers shall include stock number, nomenclature, quantity, name and address of manufacturer, and contract number

Item No CD V-110

Reference Military Specification MIL-B-15785 (BuMed) Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Standard Item Specifications

Revision 1—November 2, 1951

STOCK NUMBER 3-119-755 Unit Box

NOMENCLATURE Bottle, vacuum, *blood*, with anticoagulant, 600 cc 6's

DESCRIPTION Anticoagulant solution (120 cc in each bottle)[1,2]

Per 100 Ml of Solution

1 47 gm ± 0 15 gm dextrose USP
1 32 gm ± 0 12 gm sodium citrate USP
0 48 gm ± 0 03 gm citric acid USP
Water for injection USP qs

CONTAINER Shall be a round bottle of uncolored, clear glass with a capacity of 690 cc or ± 15 cc filled to over flowing Bottle shall be suitably stoppered with three diaphragm rubber stopper of suitable design so as to maintain throughout storage the vacuum of 27 inches of mercury Shall be in strict accor dance with the applicable paragraphs of Military Specification MIL-B-15785 (BuMed)

STERILITY Shall comply with the United States Pharmacopoeia sterility test for liquids

PACKAGING, PACKING, MARKING Six bottles constitute one unit Shall be packaged in a container of suitable size and design, so constructed with interior fittings as to protect contents adequately Unless otherwise specified, four such unit cartons shall be overpacked in an exterior container to insure safe delivery to destination Marking of individual containers shall include stock number, nomenclature quantity, name, address and lot number of manufacturer Marking of intermediate and exterior containers shall include the same information and the statements, "Glass, handle with care" and "Do not freeze", and contract number

[1] For collection of 480 cc of blood to total volume of 600 cc the NIH standard is 25 cc anticoagulant per 100 cc blood

[2] 50 cc of 4 percent sodium citrate USP should be substituted for the ACD solution when blood is collected for plasma preparation and the bottles should be ordered without airway tubes.

Item No CD 1-151

Reference Minimum Requirements National Institutes of Health Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Standard Item Specifications

STOCK NUMBER 1-598-450 Unit Package

NOMENCLATURE Blood grouping serum, anti A, dried, 75 tests with diluent Potency 60 months

DESCRIPTION Shall be dried serum containing the anti-A blood group substance (human) furnished with separate, suitably preserved diluent in sufficient quantity to reconstitute the serum to 3 cc

Shall be suitable for both slide and tube tests and shall comply with the minimum requirements of the National Institutes of Health currently in effect

Not more than 6 months of the maximum expiration dating period shall have expired at time of shipment

A sample of each lot shall be submitted to the laboratory, ASMPA, for approval by the Department of Biologic Products, Army Medical Department Research and Graduate School, before release for shipment

PACKAGING, PACKING, MARKING *Bottles*—Shall be of appropriate size and constructed of Type I or II Class, USP Containers for Injection Openings shall be such that cap dropper described below will fit bottles

Closures—Shall be plastic screw cap closures The closure for the bottle of diluent shall be fitted with inner or outer seal to prevent loss of contents by evaporation Closure liner shall be nonreactive

Hermetically Sealed Barrier for Serum Bottle—Each bottle of dried serum shall be enclosed within a hermetically sealed tube envelope, or other barrier to prevent passage of moisture vapor to the contents of the bottle

Dropper—Each unit package shall contain one screw cap rubber bulb dropper that shall fit both serum and diluent bottle The orifice of the dropper shall be constructed to deliver 24 to 32 drops per cc

Item No CD V-108

Reference *Military Specification MIL-B-15785 (BuMed), Military Medical* Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

STOCK NUMBER 3-103-610 Unit Set

NOMENCLATURE Blood recipient set, indirect blood transfusion

DESCRIPTION The recipient set shall be suitable for the administration of whole blood and shall consist of one 16-gage, ½-inch long airway canula enclosed in sleeve to protect sterility, one 18-gage, 1½-inch intravenous type recipient needle enclosed in a sleeve to maintain sterility, one filter drip device and observation tube, one 40 inch length of rubber or plastic tubing, one needle adapter, one clamp device, one instruction sheet, all in accordance with applicable paragraphs of Military Specification MIL-B-15785 (BuMed)

MATERIAL Materials in the components of the recipient set shall be in strict accordance with Military Specification MIL-B-15785, with the exception of the rubber tubing which may be transparent vinyl plastic ⅛ inch in diameter, 0 020 inch wall

STERILITY The fluid path of the recipient sets and the needles shall comply with the requirements of the United States Pharmacopeia sterility tests for solids

PACKAGING, PACKING, MARKING Each complete recipient set shall be packaged in a unit container, of suitable size and design, so constructed with interior fittings to protect the contents adequately and prevent formation of elbows in the tubing Unless otherwise specified, 288 recipient sets shall be packed with suitable intermediate packing in an exterior container of suitable size and design, so constructed as to insure safe delivery to destination Marking of interior and exterior containers shall include stock number nomenclature, quantity, name and address of manufacturer, and contract number

Item No CD V-150

Reference Minimum Requirements National Institutes of Health Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Standard Item Specifications

STOCK NUMBER 1-598-390 Unit Package

NOMENCLATURE Blood grouping serum, anti B, dried 75 tests with diluent Potency 60 months

DESCRIPTION Shall be the dried serum containing the anti-B blood group substance (human) furnished with separate, suitably preserved diluent in sufficient quantity to reconstitute the serum to 3 cc

Shall be suitable for both slide and tube tests and shall comply with the Minimum Requirements of the National Institutes of Health currently in effect

Not more than 6 months of the maximum expiration dating period shall have expired at time of shipment

A sample of each lot shall be submitted to the laboratory, ASMPA, for approval by the Department of Biologic Products, Army Medical Department Research and Graduate School, before release for shipment

PACKAGING, PACKING, MARKING *Bottles* —Shall be of appropriate size and constructed of Type I or II Class, USP Containers for Injection Openings shall be such that cap dropper described below will fit bottles

Closure —Shall be plastic screw cap closures The closure for the bottle of diluent shall be fitted with inner or outer seal to prevent loss of contents by evaporation Closure liner shall be nonreactive

Hermetically Sealed Barrier for Serum Bottle —Each bottle of dried serum shall be enclosed within a hermetically sealed tube, envelope, or other barrier to prevent passage of moisture vapor to the contents of the bottle

Dropper —Each unit package shall contain one screw cap rubber bulb dropper that shall fit both serum and diluent bottle The orifice of the dropper shall be constructed to deliver 24 to 32 drops per cc

PACKAGING, PACKING, MARKING

Unit Package.—One bottle of serum in hermetically sealed barrier, one bottle of diluent, one dropper together with instructions for use, constitute one unit. Each unit shall be packaged in a carton of appropriate size constructed in accordance with Military Specification JAN-P-133—Boxes, Set up, Paperboard. Each carton shall be adequately secured to prevent accidental opening. Unless otherwise specified, 288 unit packages shall be packed, with suitable intermediate packaging, in an exterior container of suitable size and design so constructed to insure safe delivery to destination.

Label on Bottle of Serum.—Label shall bear name of contents, expiration date, lot number, manufacturer's name and address, and such other information as required by the National Institutes of Health.

Label on Bottle of Diluent.—Label shall bear name of contents, lot number, manufacturer's name and address and such other information as required by the National Institutes of Health.

Label on Unit Package.—Shall bear the Medical Department Stock number, name of item, including *number of tests for which contents may be* used, expiration date, lot number, the statement "Store in Refrigerator at 2°-10°C. (35°-50°F)," the manufacturer's name and address and such other information as required by the National Institutes of Health.

Marking of Exterior Container.—The exterior container shall be marked with the stock number, nomenclature, quantity, expiration date, the statement "Store in Refrigerator at 2°-10° C. (35°-50°F)," the manufacturer's name, address, and lot number, and such other information as may be required by the National Institutes of Health.

Item No CD V-151

Reference Minimum Requirements National Institutes of Health

Federal Civil Defense Administration

Standard Item Specifications

Stock Number	None established Unit Package
Nomenclature	Blood grouping serum, group O (anti A and anti B) dried, 75 test with diluent Potency 60 months
Description	Shall be the dried serum containing the group O (anti A and anti B) *blood group substance* (human) furnished with separate, suitably preserved diluent in sufficient quantity to reconstitute the serum to 3 cc Shall be suitable for both slide and tube tests and shall comply with the Minimum Requirements of the National Institutes of Health currently in effect Not more than 6 months of the maximum expiration dating period shall have expired at time of shipment A sample of each lot shall be submitted to the laboratory, ASMPA, for approval by the Department of Biologic Products Army Medical Department Research and Graduate School, before release for shipment
Packaging, Packing, Marking	*Bottles*—Shall be of appropriate size and constructed of Type I or II Class, USP Containers for Injection Openings shall be such that cap dropper described below will fit bottles *Closures*—Shall be plastic screw cap closures The closure for the bottle of diluent shall be fitted with inner or outer seal to prevent loss of contents by evaporation Closure liner shall be nonreactive *Hermetically Sealed Barrier for Serum Bottle*—Each bottle of dried serum shall be enclosed within a hermetically sealed tube, envelope, or other barrier to prevent passage of moisture vapor to the contents of the bottle *Dropper*—Each unit package shall contain one screw cap rubber bulb dropper that shall fit both serum and diluent bottle The orifice of the dropper shall be constructed to deliver 24 to 32 drops per cc

PACKAGING, PACKING, MARKING

Unit Package—One bottle of serum in hermetically sealed barrier, one bottle of diluent, one dropper together with instructions for use, constitute one unit Each unit shall be packaged in a carton of appropriate size constructed in accordance with Military Specification JAN-P-133—Boxes Set-up, Paperboard Each carton shall be adequately secured to prevent accidental opening

Unless otherwise specified, 288 unit packages shall be packed, with suitable intermediate packaging, in an exterior container of suitable size and design so constructed to insure safe delivery to destination

Label on Bottle of Serum—Label shall bear name of contents, expiration date, lot number, manufacturer's name and address, and such other information as required by the National Institutes of Health

Label on Bottle of Diluent—Label shall bear name of contents, lot number, manufacturer's name and address and such other information as required by the National Institutes of Health

Label on Unit Package—Shall bear the Medical Department Stock number, name of item, including number of tests for which contents may be used, expiration date, lot number, the statement "Store in Refrigerator at 2°-10° C (35°-50° F)," the manufacturer's name and address, and such other information as required by the National Institutes of Health

Marking of Exterior Container—The exterior container shall be marked with the stock number, nomenclature, quantity, expiration date, the statement "Store in Refrigerator at 2°-10° C (35°-50° F)," the manufacturer's name, address, and lot number and such other information as may be required by the National Institutes of Health

Reference Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER 1-581-980 Unit Bottle

NOMENCLATURE Albumin, serum, bovine, 30 percent, 20 cc

DESCRIPTION Shall be concentrated bovine albumin suitable for use in the determination of the Rh factor in human sera and in crossmatching, conforming to the following requirements

1 Protein 30 percent ± 2 percent (fraction V)
2 Preservative 0 1 percent (acetyl tryptophane)
3 pH 6 9 ± 0 2
4 Freezing point −0 4° C to −0 65° C

Shall be processed by a method approved by the Director of the Plasma Foundation Control Laboratory, Department of Physical Chemistry, Harvard Medical College, Boston, Mass

PACKAGING, PACKING, MARKING Each unit shall be packaged in a narrow-mouthed glass bottle with glass conforming to Type I or II, USP (latest revision) Containers for Injection Closure shall be rubber diaphragm stopper with adequate aluminum seal Unless otherwise specified, 288 bottles shall be packed with suitable intermediate packaging in exterior containers of suitable size and design and so constructed as to insure safe delivery to destination Each bottle shall be marked with the stock number, nomenclature, quantity, date of manufacture the statement, "Keep under refrigeration 36° to 50° F (2° to 10° C)," manufacturer's name, address, and lot number The intermediate and exterior containers shall be marked with stock number, nomenclature, quantity, manufacturer's name address and lot number, and contract number

PACKAGING, PACKING, MARKING

Unit Package —One bottle of serum in hermetically sealed barrier, one bottle of diluent, one dropper together with instructions for use, constitute one unit Each unit shall be packaged in a carton of appropriate size constructed in accordance with Military Specification JAN–P–133—Boxes, Set-up, Paperboard Each carton shall be adequately secured to prevent accidental opening

Unless otherwise specified, 288 unit packages shall be packed, with suitable intermediate packaging, in an exterior container of suitable size and design so constructed to insure safe delivery to destination

Label on Bottle of Serum —Label shall bear name of contents, expiration date, lot number, manufacturer s name and address and such other information as required by the National Institutes of Health

Label on Bottle of Diluent —Label shall bear name of contents, lot number manufacturer s name and address and such other information as required by the National Institutes of Health

Label on Unit Package —Shall bear the Medical Department stock number, name of item, including number of tests for which contents may be used expiration date, lot number, the statement "Store in Refrigerator at 2°–10°C (35°–50°F)," the manufacturer s name and address, and such other information as required by the National Institutes of Health

Marking of Exterior Container —The exterior container shall be marked with the stock number, nomenclature, quantity, expiration date, the statement "Store in Refrigerator at 2°–10°C (35°–50°F)," the manufacturer's name, address, and lot number, and such other information as may be required by the National Institutes of Health

Item No CD 1-131

Reference: Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER 1-581-980 Unit Bottle

NOMENCLATURE Albumin, serum, bovine, 30 percent, 20 cc

DESCRIPTION Shall be concentrated bovine albumin suitable for use in the determination of the Rh factor in human sera and in crossmatching, conforming to the following requirements

1 Protein 30 percent ± 2 percent (fraction V)
2 Preservative 0 1 percent (acetyl tryptophane)
3 pH 6 9 ± 0 2
4 Freezing point −0 4° C to −0 65° C

Shall be processed by a method approved by the Director of the Plasma Foundation Control Laboratory, Department of Physical Chemistry, Harvard Medical College, Boston, Mass

PACKAGING, PACKING, MARKING Each unit shall be packaged in a narrow-mouthed glass bottle with glass conforming to Type I or II, USP (latest revision) Containers for Injection Closure shall be rubber diaphragm stopper with adequate aluminum seal Unless otherwise specified, 288 bottles shall be packed with suitable intermediate packaging in exterior containers of suitable size and design and so constructed as to insure safe delivery to destination Each bottle shall be marked with the stock number nomenclature, quantity, date of manufacture the statement, "Keep under refrigeration 36° to 50° F (2° to 10° C)," manufacturer's name, address, and lot number The intermediate and exterior containers shall be marked with stock number, nomenclature, quantity, manufacturer s name address and lot number, and contract number

Item No CD V-130

Reference *Rosin's Reagent Chemicals and Standards Military Medical* Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER 1-161-525 Unit Bottle

NOMENCLATURE Cupric Sulfate, 170 gm Special reagent for measuring specific gravity for blood

DESCRIPTION Shall be copper sulfate pentahydrate ($Ca\ SO_4\ 5H_2O$) conforming to Rosin's Reagent Chemicals and Standards, except that the loss on drying at 300° C to 350° C shall be not less than 36 percent and not more than 36 14 percent and that the material shall be in the form of fine crystals Each bottle shall contain not less than 169 9 gm and not more than 170 1 gm of pure copper sulfate pentahydrate

PACKAGING, PACKING, MARKING The specified amount of salt shall be placed in a wide mouth glass bottle of appropriate size and design then stoppered with a suitable closure The closure shall then be completely sealed with microcrystalline wax to prevent passage of any water vapor Unless otherwise specified forty eight (48) bottles shall be packed in a shipping container of suitable size and design so constructed to insure safe delivery to destination Marking on individual bottle shall include stock number, nomenclature, quantity, manufacturer's name, address and lot number, also the contract number In addition the individual bottle label shall bear the following

Amounts of water recommended for preparing a solution with a specific gravity of 1 100
(Revised January 1, 1946)

Temperature centigrade	Ml H_2O
10 -17	1004
17 -22	1005
22 -26	1006
26 -29	1007
29 -33	1008
33°-36	1009
36°-38	1010
38 -40	1011

Item No CD 3-132

Reference Federal Specification GG-K-516 Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER 3-447-120 Unit Packaging

NOMENCLATURE Blade, operating knife, No 11, 6's

DESCRIPTION Shall be suitable for use as a lancet and shall be size 1, Type CC, 6's, in accordance with Federal Specification GG-K-516

MATERIAL Blades shall be of steel properly hardened and tempered, and of one-piece construction Blades shall comply with the applicable design and dimensions shown in figures 4a to 4d and 5a to 5d, inclusive, Federal Specification GG-K-516 for the various sizes and types That part of the blade which is adjacent to the perforation and which fits into the groove of the nose of the handle shall be 0 0145 to 0 0155 inch thick The remainder of the blade shall be 0 0145 to 0 212 inch thick, except that it may be thicker along the back edge as a means of reinforcement Blades shall fit accurately, shall be easily detachable from the handles for which they are designed, and shall be interchangeable with blades of the same size

The cutting edge of blade shall be central and uniform There shall be no break or jag in the edge, and it shall be free of nicks and feathers The cutting edge shall be surgically sharp and shall have an included angle not greater than 24°

FINISH All edges of the blade, except cutting edge, shall be smooth and free from burrs Blades shall be so finished as to remove pits and grind marks Blades shall show no corrosion and shall be protected from corrosion by a film of oil, paraffin, or other suitable coating

PACKAGING, PACKING, MARKING

Each unit (6 blades) shall be wrapped in acid free, noncorrosive paper of a pH of not less than 6.5 nor more than 7.5, in such a manner that no blade will be in direct contact with another. Each package of 6 blades shall be processed in accordance with *Method IA, Military Specification* JAN-P-116, using technique E2c, Method IA8, Appendix IV or equally protective method. Unless otherwise specified, 1440 unit packages of 6 blades each shall be packed in a shipping container, using adequate interior packing in such a manner as to insure safe delivery to *destination. Marking of exterior and interior* containers shall include stock number, nomenclature, quantity contained therein, manufacturer's name and address, and contract number.

Item No CD 1-156

Reference Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Standard Item Specifications

STOCK NUMBER	None established Unit Each
NOMENCLATURE	Container, blood, shipping
DESCRIPTION	The blood shipping container shall be a specially constructed, insulated, hinge cover box, in strict accordance with the latest purchase specifications supplied by the Armed Services Medical Procurement Agency for the subject item The box shall hold 24 units of whole blood between the temperatures of 39° F and 50° F for a period of 24 hours with a single icing The box, plus 24 bottles of whole blood, should not weigh over 130 pounds and it shall occupy between 6 to 8 cubic feet of space The box shall be fitted with suitable interior fittings to provide protection against breakage of contents The box shall provide suitable space for ice to permit compliance with temperature tests
TESTS	The box and its performance shall comply with all tests prescribed by the Armed Services Medical Procurement Agency specifications and purchase descriptions
PACKAGING, PACKING, MARKING	The box itself need not be over-packed for shipping Marking shall be that specified in the invitation to bid

PACKAGING, PACKING, MARKING

Each unit (6 blades) shall be wrapped in acid free, noncorrosive paper of a pH of not less than 6 5 nor more than 7 5, in such a manner that no blade will be in direct contact with another Each package of 6 blades shall be processed in accordance with Method IA, Military Specification JAN-P-116, using technique E2c, Method IA8, Appendix IV or equally protective method Unless otherwise specified, 1440 unit packages of 6 blades each shall be packed in a shipping container, using adequate interior packing in such a manner as to insure safe delivery to destination Marking of exterior and interior containers shall include stock number, nomenclature, quantity contained therein, manufacturer's name and address, and contract number

Item No CD V-142

Reference National Institutes of Health Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER 1-607-070 Unit Package

NOMENCLATURE Plasma, normal human, dried, irradiated, commercial, 500 cc

DESCRIPTION Shall be complete injection assembly with diluent Plasma and diluent shall be in accordance with the latest Minimum Requirements of the National Institutes of Health

AGE LIMITATION Not more than 6 months of the maximum 5 year expiration dating period of the plasma shall have expired at time of delivery

PACKAGING, PACKING, MARKING The dried plasma, the diluent, the injection and airway assemblies, including the intravenous needle, the hose-hub needle, the double ended needle, shall be packaged in standard commercial containers with adequate interior fittings to prevent shifting and breakage of contents Packaging may also be in hermetically sealed tins within a sealed fiber board carton in accordance with the latest Military Medical Purchase Descriptions of the Armed Services Medical Procurement Agency with the stock number references, if so specified in the invitation for bids Unless otherwise specified, twelve such complete units shall be packed in an exterior shipping container of suitable size and design, so constructed to insure safe delivery to destination Marking of the unit package and its interior component packages when such package is in accordance with commercial standards, shall include stock number, nomenclature, manufacturer's name, address and lot number, date of manufacture, and such other information required by the National Institutes of Health, together with complete instructions for reconstitution, assembly, and use Marking of the unit package and its interior component package when such package is in accordance with the latest Military Medical Purchase Description,

Item No CD V-111

Reference National Institutes of Health Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

STOCK NUMBER 1-607-075 Unit Package

NOMENCLATURE Plasma, normal human, dried, irradiated, donated, 500 cc

DESCRIPTION Shall be complete injection assembly with diluent Plasma and diluent shall be in accordance with the latest Minimum Requirements of the National Institutes of Health

AGE LIMITATION Not more than 6 months of the maximum 5 year expiration dating period of the plasma shall have expired at time of delivery

PACKAGING, PACKING, MARKING The immediate packaging of the plasma and diluent, the injection and air-way assemblies, including all needles, the placing of the immediate containers of plasma, diluent, and all components in hermetically sealed cans and the placing of the complete unit in one sealed fiber board carton, shall be in strict accordance with the latest Military Medical Purchase Description of the Armed Services Medical Procurement Agency for the stock number references Unless otherwise specified, 12 such complete units shall be packed in an exterior shipping container of suitable size and design, so constructed as to insure safe delivery to destination Marking of the unit package and its interior component package shall be in accordance with the purchase description herein referenced Marking of the exterior container shall include stock number, nomenclature, quantity, expiration date, name, address, and lot number of manufacturer, contract number and the statements "Subject to damage by freezing" and "Does not require refrigeration"

Item No CD 1-159

Reference National Research Council Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Standard Item Specifications

STOCK NUMBER 1-161-890 Unit Package

NOMENCLATURE Dextran injection, 6 percent, 500 cc

DESCRIPTION Dextran injection Dextran injection shall be a sterile, nonpyrogenic solution of a specially hydrolyzed dextran produced by the propagation of a strain of Leuconostoc It shall not interfere with direct blood matching or with typing for blood group or Rh factor by the microscope slide technique when injected intravenously It shall contain no preservative The solution shall be stable, clear, colorless or faint yellow, odorless, and nontoxic The diluent shall be isotonic sodium chloride solution It shall comply with the requirements of the National Research Council and conform to the latest revision of the Military Medical Purchase Description issued by the Armed Services Medical Procurement Agency

Bottle assembly, including closure Shall consist of the bottle, airway tube, suspension apparatus, and closure in accordance with specifications and drawings issued by the Armed Services Medical Procurement Agency

Injection apparatus The injection apparatus shall be suitable for the administration of the dextran solution The apparatus shall be nontoxic, sterile and pyrogen free when tested in accordance with the applicable tests contained in these specifications, and shall consist of a glass or plastic housing incorporating a drip device, a 17-gage, 1½ inch canula intravenous needle airway canula a length of plastic tubing and a clamping device for regulating the flow of liquid through the set by compression of the tubing, all in accordance with the Military Medical Purchase Description issued by the Armed Services Medical Procurement Agency

MATERIAL All materials which make up the components of the dextran injection package shall be in strict accordance with the latest revision of the Military

Packaging,
Packing,
Marking

shall likewise follow the marking specified in that Purchase Description In both cases, marking of the exterior container shall include stock number, nomenclature, quantity, expiration date, name, address, and lot number of manufacturer, contract number and the statements, "Subject to damage by freezing," and "Does not require refrigeration "

PACKAGING, PACKING, MARKING

injection apparatus is included therein Intermediate containers shall be marked to identify contents The exterior container shall be marked to show stock number, nomenclature, quantity of contents, date of manufacture, manufacturer's name, address, and lot number, and the statements, "Store in a cool place," and "Do not freeze"

MATERIAL Medical Purchase Description issued by the Armed Services Medical Procurement Agency

VACUUM Each filled bottle shall have a vacuum of not less than 15 inches of mercury mechanically induced in the flask before stoppering and autoclaving

STERILITY AND FREEDOM FROM HYDROGENS The dextran injection, the fluid path of the injection apparatus, including the intravenous needle and the airway canula, shall be sterile and free from pyrogen when tested in accordance with methods prescribed in the Military Medical Purchase Description

WORKMANSHIP The dextran injection and the injection apparatus shall be free from any defects which detract from their appearance or might impair their serviceability

PACKAGING, PACKING, MARKING The dextran injection and the complete injection apparatus shall be packaged in a carton of suitable size and design, fitted with interior fittings to prevent any damage to the contents which will interfere with the full efficiency of the solution or the administering equipment Unless otherwise specified, 24 unit packages, each containing 1 bottle of dextran solution and 1 administration set, shall be packed, with suitable intermediate packing, in an exterior container of suitable size and design, so constructed as to insure safe delivery to destination Marking of bottle shall include stock number, nomenclature, quantity, manufacturer s name, address, and lot number, date of manufacture, the words, ' Sterile," and "Non-Pyrogenic," and such other information required by Federal and State laws In addition, the label shall bear the following statements This material acceptable for use only if substantially free of any turbidity or undissolved material which can be readily detected without accessory magnification" and "Do not use if no vacuum is detectable " The label shall also bear instructions for use and the statements "Store in a cool place " ' Do not permit to freeze," and ' For stockpiling or emergency use only " Each carton containing the bottle of dextran solution and the injection apparatus shall be marked with the same information included on the bottle, plus a statement to the effect that the

Material All materials which make up the components of the PVP injection package shall be in strict accordance with the latest revision of the Military Medical Purchase Description issued by the Armed Services Medical Procurement Agency

Vacuum Each filled bottle shall have a vacuum of not less than 15 inches of mercury, mechanically induced in the flask before stoppering and autoclaving

Sterility and Freedom from Pyrogens The PVP injection, the fluid path of the injection apparatus, including the intravenous needle and the airway canula, shall be sterile and free from pyrogen when tested in accordance with methods prescribed in the Military Medical Purchase Description

Workmanship The PVP injection, container, and the injection apparatus shall be free from any defects which detract from their appearance or might impair their serviceability

Packaging, Packing and Marking The PVP injection and the complete injection apparatus shall be packaged in a carton of suitable size and design, fitted with interior fittings to prevent any damage to the contents which will interfere with the full efficiency of the solution or the administering equipment Unless otherwise specified, twenty four unit packages, each containing one bottle of PVP injection and one administration set, shall be packed, with suitable intermediate packing, in an exterior container of suitable size and design, so constructed to insure safe delivery to destination Marking of bottle shall include stock number, nomenclature, quantity, manufacturer's name, address and lot number, date of manufacture, the words, "Sterile," and "Non-Pyrogenic," and such other information required by Federal and State laws In addition, the label shall bear the following statements "This material acceptable for use only if substantially free of any turbidity or undissolved material which can be readily detected without accessory magnification" and "Do not use if no vacuum is detectable " The label shall also bear instructions for use and the statements, "Store in a

Item No CD V-165

Reference National Research Council Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Standard Item Specifications

STOCK NUMBER NS-1 Unit Package

NOMENCLATURE Polyvinyl pyrrolidone injection, M-1, 3 5%, 500 cc

DESCRIPTION Polyvinyl pyrrolidone injection The polyvinyl pyrrolidone injection, herein designated as PVP injection, shall be a sterile, nonpyrogenic solution of approximately 3 5 grams of PVP in 100 ml of physiological saline solution It shall not interfere with direct blood matching or with typing for blood group or Rh factor by the microscope slide technique when injected intravenously It shall contain no preservative The solution shall be stable, clear, colorless or light amber, practically odorless, and nontoxic The diluent shall be isotonic sodium chloride solution It shall comply with the requirements of the National Research Council and conform to the latest revision of the Military Medical Purchase Description issued by the Armed Services Medical Procurement Agency

Bottle assembly, including closure Shall consist of the bottle, airway tube, suspension apparatus, and closure in accordance with specifications and drawings issued by the Armed Services Medical Procurement Agency

Injection apparatus The injection apparatus shall be suitable for the administration of the PVP injection The apparatus shall be nontoxic, sterile and pyrogen free when tested in accordance with the applicable tests contained in these specifications, and shall consist of a glass or plastic housing incorporating a drip device, a 17 gage 1½ inch canula intravenous needle, airway canula, a length of plastic tubing and a clamping device for regulating the flow of liquid through the set by compression of the tubing, all in accordance with the Military Medical Purchase Description issued by the Armed Services Medical Procurement Agency

MATERIAL All materials which make up the components of the PVP injection package shall be in strict accordance with the latest revision of the Military Medical Purchase Description issued by the Armed Services Medical Procurement Agency

VACUUM Each filled bottle shall have a vacuum of not less than 15 inches of mercury, mechanically induced in the flask before stoppering and autoclaving

STERILITY AND FREEDOM FROM PYROGENS The PVP injection, the fluid path of the injection apparatus, including the intravenous needle and the airway canula, shall be sterile and free from pyrogen when tested in accordance with methods prescribed in the Military Medical Purchase Description

WORKMANSHIP The PVP injection, container, and the injection apparatus shall be free from any defects which detract from their appearance or might impair their serviceability

PACKAGING, PACKING AND MARKING The PVP injection and the complete injection apparatus shall be packaged in a carton of suitable size and design, fitted with interior fittings to prevent any damage to the contents which will interfere with the full efficiency of the solution or the administering equipment Unless otherwise specified, twenty-four unit packages, each containing one bottle of PVP injection and one administration set, shall be packed, with suitable intermediate packing, in an exterior container of suitable size and design, so constructed to insure safe delivery to destination Marking of bottle shall include stock number, nomenclature, quantity, manufacturer's name, address and lot number date of manufacture, the words, "Sterile," and "Non-Pyrogenic,' and such other information required by Federal and State laws In addition the label shall bear the following statements "This material acceptable for use only if substantially free of any turbidity or undissolved material which can be readily detected without accessory magnification" and "Do not use if no vacuum is detectable " The label shall also bear instructions for use and the statements, "Store in a

cool place," "Do not permit to freeze," and "War Reserve—For Emergency Use Only" Each carton containing the bottle of PVP injection and the injection apparatus shall be marked with the same information included on the bottle, plus a statement to the effect that the injection apparatus is included therein Intermediate containers shall be marked to identify contents The exterior container shall be marked to show stock number, nomenclature, quantity of contents, date of manufacture, manufacturer's name, address and lot number, and the statements, 'Store in a cool place," "Do not freeze" and War Reserve—For Emergency Use Only'

Item No CD V-123

Reference Military Specification MIL-B-15785 (BuMed) Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Standard Item Specifications

STOCK NUMBER 3-119-790 Unit Box

NOMENCLATURE Bottle, vacuum, blood, with dextrose, 2,000 cc 6's

DESCRIPTION Shall be a round bottle of uncolored clear glass suitable for pooling plasma or serum measuring approximately 4½ inches in diameter and approximately 13 inches in height, containing 250 cc of sterile solution of dextrose injection USP with 50 percent dextrose The bottle shall have a capacity of approximately 2,350 cc filled to overflowing and shall be graduated in intervals of 100 cc from bottom to top to a volume of 2,000 cc in upright positions It shall be graduated in intervals of 100 cc to show quantity delivered in inverted position from 0 to 2 000 cc It shall be stoppered with a three diaphragm coated rubber stopper having a glass inner airway of adequate length fitted to one of the diaphragms The rubber stopper shall be fastened securely with a three piece tear-off aluminum seal and shall be otherwise protected to keep the top surface of the diaphragms sterile till ready for use The bottom of the bottle shall be fitted with a bail for suspending bottle in the inverted position

MATERIAL Glass—Bottle and airway shall be of Type I or Type IV clear glass conforming to the USP (latest revision) Containers for injection Rubber Stopper—Shall conform to paragraph 3 2 1 2 of Military Specification MIL B-15785

VACUUM Each bottle shall be evacuated to a negative pressure of not less than 27 inches of mercury at a temperature of 75° ± 2° F

STERILITY AND PYROGENITY The interior of the bottle, the contents, and entire rubber stopper shall be sterile and pyrogen free when tested in accordance with the applicable paragraphs of the USP latest revision

PACKAGING, PACKING MARKING Six bottles shall constitute one unit and shall be packaged in a container of suitable size and design, so constructed with interior fittings as to adequately protect the contents Unless other-

PACKAGING, PACKING, MARKING

wise specified one such carton (1 unit) shall be overpacked in an exterior container to insure safe delivery to destination Marking of individual containers shall include stock number, nomenclature, name, address, and lot number of manufacturer and such other information as may be required Label shall also provide space for recording the No ------, Pool ------, Serology ------, Date of Collection ------, and Recipient ----- The intermediate and exterior containers shall be marked with the stock number, nomenclature, quantity, name address and lot number of manufacturer, and the contract number

Item No CD 1-121

Reference Military Specification MIL-B-15785 (BuMed) Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Standard Item Specifications

STOCK NUMBER 3-119-730 Unit Box

NOMENCLATURE Bottle, vacuum, blood, 500 cc 6's

DESCRIPTION Shall be a round bottle of uncolored clear glass with a capacity of 700 cc ±25 cc filled to overflowing Bottle shall be suitably stopped with three diaphragm rubber stopper of suitable design so as to maintain throughout storage the vacuum of 27 inches of mercury It shall be in strict accordance with the applicable paragraphs of Military Specification MIL-B-15785 (BuMed) except that the bottle shall contain no solution

STERILITY Shall comply with the requirements of the United States Pharmacopoeia sterility tests

PACKAGING, PACKING, MARKING Six bottles shall constitute one unit and shall be packaged in a container of suitable size and design, so constructed with interior fittings as to adequately protect contents Unless otherwise specified, four such unit cartons shall be overpacked in an exterior container to insure safe delivery to destination Marking of individual container shall include stock number, nomenclature, name, address, and lot number of manufacturer, and such other information as may be required Label shall also provide space for recording the Date ----------, Pool ----------, No ------, Serology ------, Recipient---------- The intermediate and exterior containers shall be marked with the stock number, nomenclature, quantity, name address, and lot number of the manufacturer, and the contract number

Reference Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER 3-103-000 Unit Set

NOMENCLATURE Blood aspirating donor set

DESCRIPTION Shall be a blood aspirating donor set for indirect blood transfusion and plasma pooling Set shall consist of flow valve assembly with puncturing needle, rubber tubing donor needle in glass vial, and six (6) spare rubber flow valve-washers similar to Baxter No LD, plus six (6) spare rubber flow valve washers, as illustrated on page XII of American Hospital Supply Corporation Catalog

PACKAGING, PACKING, MARKING Each unit shall be packaged with suitable interior fittings to insure against shifting and breakage in a carton of appropriate size, constructed in accordance with JAN-P-133 Unless otherwise specified, 72 sets shall be packed, with suitable intermediate packaging, in an exterior container of suitable size and design so constructed as to insure safe delivery to destination Marking of interior and exterior packages shall include stock number, nomenclature quantity, name and address of manufacturer, and contract number

Item No CD V-126 and 127

Reference Military Medical Purchase Description (latest revision) Armed Services Medical Procurement Agency Drawing No 1013

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER 3-486-900 Unit Each
3-487-100

NOMENCLATURE Needle, aspirating, 13 gage, 10 inch
Needle, aspirating, 13 gage, 15 inch

DESCRIPTION Shall be an aspirating needle, suitable for pooling plasma in blood banks Canula shall terminate with a sharp bevel, the lumen shall be closed flush with the bevel and the lower opening shall be approximately 4 mm from the end of the canula Shall be as shown on Armed Services Medical Procurement Agency drawing No 1013, dated February 5, 1946

MATERIAL Shall be in strict accordance with Federal Specification GG-I-526a, dated April 8, 1949 Canula shall be Class 2, Composition G (c) Hub shall be Class 4, Composition N Hardness (par 3 9) shall be Rockwell C35-C40

PACKAGING, PACKING, MARKING Each needle shall be thoroughly cleaned in accordance with the applicable method as outlined in Appendix I of Military Specification JAN-P-116 Each needle shall then be completely wrapped in neutral tissue or other suitable anticorrosive material and then packaged in a carton of appropriate size constructed in accordance with Jan-P-120 or JAN-P-133, with sufficient cushion material to prevent damage to the needle Unless otherwise specified 288 needles of like size shall be packed with suitable interior packaging, in an exterior container of suitable size and design so constructed to insure safe delivery to destination Marking of interior and exterior containers shall include stock number, nomenclature, quantity, name and address of manufacturer, and contract number

Item No CD V-125

Reference Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER 3-103-000 Unit Set

NOMENCLATURE Blood aspirating donor set

DESCRIPTION Shall be a blood aspirating-donor set for indirect blood transfusion and plasma pooling Set shall consist of flow valve assembly with puncturing needle, rubber tubing donor needle in glass vial, and six (6) spare rubber flow valve washers, similar to Baxter No LD, plus six (6) spare rubber flow valve-washers, as illustrated on page XII of American Hospital Supply Corporation Catalog

PACKAGING, PACKING, MARKING Each unit shall be packaged with suitable interior fittings to insure against shifting and breakage in a carton of appropriate size, constructed in accordance with JAN-P-133 Unless otherwise specified, 72 sets shall be packed, with suitable intermediate packaging, in an exterior container of suitable size and design so constructed as to insure safe delivery to destination Marking of interior and exterior packages shall include stock number, nomenclature, quantity, name and address of manufacturer, and contract number

Reference Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER 1-472-690 Unit Bottle

NOMENCLATURE Thioglycollate fluid medium, 1 lb

DESCRIPTION Shall be a stable dehydrated powder and shall be in accordance with revised formula for the sterility testing of biologicals as required by the National Institutes of Health as follows

	Grams
Pancreatic digest of casein	15 0
1 Cystine	0 75
Dextros anhydrous	5 0
Water soluble yeast extract	5 0
Sodium chloride	2 5
Sodium thioglycollate	0 5
Resazurin	0 001
Agar, granulated	0 75

The ingredients used shall comply with the requirements of the USP or NF if described therein if not described therein, each shall be of suitable bacteriological grade

Twenty-nine and one half (29 5) grams of the above formula shall make 1 liter of finished medium When prepared in accordance with the manufacturer s labeled directions, 1 pound shall yield not less than 15 4 liters of medium with an approximate final pH of 7 1

PACKAGING, PACKING, MARKING Each unit (1 lb) of Thioglycollate Fluid Medium shall be packaged in a wide mouthed light resistant glass bottle with screw-cap closure The closure shall be phenol resin plastic or metal, treated inside and out to prevent corrosion Closure liner shall be nonreactive, each closure shall be sealed adequately to prevent passage of moisture vapor Unless otherwise specified, twenty four (24) bottles shall be packed in an exterior container of suitable size and design so constructed to insure safe delivery to destination Marking of intermediate container shall include stock number, nomenclature, quantity, formula,

Item No CD V-131 and 128

Reference Federal Specification No GG-S-921a and amendment 2 Military Medical Purchase Description (latest revision)

FEDERAL CIVIL DEFENSE ADMINISTRATION

Purchase Description

Standard Item Specifications

STOCK NUMBER	3-803-800
	None established Unit Each
NOMENCLATURE	Syringe, Luer, 2 cc
	Syringe, Luer, 50 cc
DESCRIPTION	Shall be Luer-slip glass, Type I, 2 cc or Type II 50 cc, whichever is specified in accordance with Federal Specification GG-S-921a The 2 cc syringe shall be graduated to $^1/_{10}$ of a cc The 50 cc syringe shall be graduated to 1 cc Tip shall be concentric to axis of the barrel
MATERIAL	Shall be highest grade heat resistant, corrosion resistant glass throughout tubing for the plunger, sizes 5 cc and over and either rod or tubing for sizes under 5 cc, the glass shall have a density of not more than 2 4
WORKMANSHIP	Shall be first class in every respect free from any defect affecting appearance or which may affect serviceability
PACKAGING, PACKING, MARKING	Each syringe shall be packaged in a set up box of suitable size and design fitted with interior fittings to prevent shifting and breakage Unless otherwise specified, 360 2 cc syringes, Item Number 3-803-800 or 144 50 cc syringes, shall be packed with suitable intermediate packaging in an exterior container of suitable size and design so constructed as to insure safe delivery to destination Marking of interior and exterior containers shall *include stock number, nomenclature,* quantity manufacturer's name and address and contract number

STANDARD OPERATING PROCEDURES FOR COLLECTION AND PROCESSING OF WHOLE BLOOD

The procedures detailed in this supplement have been developed for joint use in the national defense program by the American National Red Cross with the assistance of the Department of Defense and the Federal Civil Defense Administration, and have the concurrence of the Laboratory of Biologics Control of the National Institutes of Health The application of the standard procedures has been adapted here for use specifically in civil defense emergencies The employment of standard operating procedures by all blood banks and donor centers for the collection and processing of blood in civil defense emergencies is essential for maximum utilization of whole blood resources throughout the country

This supplement describes standards of selection of blood donors and techniques used in the collection of blood, methods of storage, labeling and shipping of blood, and laboratory techniques for the processing of whole blood, determination of blood groups, group confirmation, Rh type determinations, and serology tests

The Donor

Medically there are two responsibilities in regard to the collection of blood for transfusion The standards of selection must be such that (1) the specified amount of blood can be removed without harm to the donor and (2) the blood so removed must not be harmful to the recipient. To achieve this, the following professional personnel are required

(*a*) A licensed physician should always be present in the blood bank or at the mobile unit operation when blood is being collected and when donors are resting following donation

(*b*) Registered nurses or properly qualified and trained technicians should take the medical history, determine the hemoglobin level and perform the *venesection* They are usually regular employees Properly qualified and trained volunteer registered nurses or technicians may also carry out these functions Volunteer nurses aides who are specially trained may assist in these operations

PACKAGING, PACKING, MARKING

directions for preparation of Medium for use, manufacturer's name, address, and lot number Marking of exterior container shall include stock number, nomenclature, quantity, name, address, lot number of manufacturer, and contract number

99.6° F by mouth taken at proper interval after smoking or the ingestion of hot or cold liquids should exclude a donor

Pulse—Should be normal Rates less than 60 or above 100 and any irregularity of rhythm should be referred to the medical director

Blood pressure—Should be between 100 and 200 mm Hg systolic and less than 100 mm Hg diastolic. Diastolic readings checked by the physician and found to be between 100 and 110 mm Hg may be accepted at the discretion of the physician The diastolic reading is taken in the second phase

Hemoglobin—The level must be 12.5 grams percent or higher as determined by the Phillips & Van Slyke $CuSO_4$ method using a solution with a specific gravity of 1.053 Directions for performing the hemoglobin determinations will be found on page 132

Medical history—Consideration should be given to each of the following factors

(a) Illness in last month Particular note should be made of the presence or repeated occurrence of upper respiratory infections No arbitrary limit should be set regarding the interval after which the donor becomes eligible for venipuncture, because of the variability, severity, and duration of such infections

Donors with chronic sinusitis and hay fever are acceptable if they are not in an acute stage and are otherwise in good health

Detailed information should be obtained from donors who give a history of severe septic sore throat within the preceding 3 months, and they should be accepted only after consultation with the physician

(b) Surgical operation in the past 6 months (oral surgery included) An affirmative answer should be referred to the physician Prolonged convalescence or blood or plasma transfusions during hospitalization are usually causes for exclusion

(c) During pregnancy and for 12 months postpartum donors are excluded Miscarriage within the last 12 months should be referred to the physician

(d) Malaria Persons who have ever had malaria or have had intensive suppressive therapy should not be accepted as donors for whole blood transfusion Those who have a history of malaria or who have received intensive suppressive therapy but have had no clinical attack in the preceding 2 years may be accepted for donations to be used in the preparation of plasma (or plasma fractions) The medical director may exercise discretion in accepting persons who have received light seasonal courses of quinine in the Southern United States but who have never had clinical infection

(e) Undulant fever Donors with a history of undulant fever may be accepted if they have had no attack in the preceding 2 years

Selection of the Donor

Experience has shown that the following criteria are adequate to protect the donor's health and welfare, and to provide a safe bottle of blood If the local medical authorities believe that changes are in order, these should be recommended to FCDA before being put into effect for use in civil defense emergencies Similarly, changes may be recommended by FCDA on the advice of competent medical authority

Donor Registration Card

The donor registration card is the permanent record of the care with which a donor is selected It is a medical record and the medical director of each blood bank is responsible for its confidential custody Only properly qualified personnel should have access to it and it should be kept on permanent file

Physicians, nurses or technicians who fill out the donor registration card should take care to see that the prospective donor understands the questions Every question should be asked and the proper answer recorded each time a person presents himself for donation no matter how many times he may have donated before

Medical Criteria for Selection

Interval between donations—Must be at least 8 weeks but no one should be accepted more often than five times a year

Sex—Both male and female donors are acceptable

Race—Members of all races are acceptable

Age—Persons 18 through 59 are acceptable Unmarried persons not legally of age must present the written consent of a parent or guardian to be accepted Married minors and minors on active duty in the Armed Services *do not require a release from parent, guardian,* spouse, or Commanding Officer Legal age of majority is that of the State in which blood is being collected

Weight—Prospective donors should weigh at least 110 pounds to give 500 cc of blood Persons weighing between 100 and 110 pounds and extremely desirous to donate may be allowed to give 250 to 500 cc. of blood upon the approval of the medical director No arbitrary maximum limit is set on weight. This is left to the discretion of the medical director The donor should be questioned concerning recent changes in weight Any marked change should be brought to the attention of the physician in charge.

Temperature—Should be normal. The medical director must decide what limits of normal variation are allowable depending on environmental conditions at time of selection. A temperature over

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Medical Criteria for Selection

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Sex—Both male and female donors are acceptable

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Weight—Prospective donors should weigh at least 110 pounds to give 500 cc of blood Persons weighing between 100 and 110 pounds and extremely desirous to donate may be allowed to give 250 to 500 cc of blood upon the approval of the medical director No arbitrary maximum limit is set on weight This is left to the discretion of the medical director The donor should be questioned concerning recent changes in weight Any marked change should be brought to the attention of the physician in charge.

Temperature—Should be normal. The medical director must decide what limits of normal variation are allowable depending on environmental conditions at time of selection A temperature over

(2) Smallpox Donors may be accepted 2 weeks after immuno reaction or when the scab comes off

(3) Typhoid, typhus, Rocky Mountain spotted fever, influenza, yellow fever or cholera, diptheria or tetanus. Donors may be accepted 2 weeks after last injection

(4) Vitamin, hormone, liver and other injections Donors should be evaluated by the medical director on an individual basis

(i) **Occupation** Flight crews of commercial planes and members of the Armed Services who will participate in flight operations within 14 days should not be accepted Persons who operate heavy equipment such as power machinery, cranes, buses, or trains, or climb ladders and scaffolds or who are engaged in similar occupations hazardous to themselves or others should be deferred if they must return to these occupations within 12 hours after donation

(j) **General** The final responsibility for the acceptance or rejection of a donor rests with the physician in charge of an operation Even though a donor meets the minimum requirements, he may be refused if the physician deems him a "poor risk" Every donor deemed not acceptable for medical reasons should be seen by the physician.

Nourishment

Donors should be instructed to omit all fats and fatty foods such as butter, oleomargarine, salad dressing, cream, ice cream, whole milk, eggs, cheese, fat meats, pork, bacon, soups (except for clear consomme) during a 4 hour period before donating blood It should be emphasized that it is wise for donors to take some nourishment in the form of fruit juice, tea or coffee without cream, plain crackers or dry toast without butter, but with jelly, etc, shortly before coming to the blood bank Too long a period of strict fasting lowers the blood sugar and increases the reaction rate Prospective donors who have eaten a heavy fatty meal within 4 hours should be rejected if the circumstances of the emergency permit

Before entering the donor room, donors should be provided with a sweetened drink to increase their blood sugar level Light nourishment should be provided for donors after donation This improves public relations of the blood bank, lessens the reaction rate of the donor, and gives him an activity so that the period of post donation observation may be prolonged without making the donor impatient Warm or cool liquid refreshment, preferably with some carbohydrate content, and some simple food are sufficient Elaborate sandwiches and snacks are to be avoided They are unnecessary, expensive, and may induce reactions by overloading the stomach

(*f*) **Jaundice**

(1) A history of viral hepatitis (infectious or homologous type) at any time in the past automatically excludes a donor (except when blood is to be used for plasma fractions) This is a requirement of the National Institutes of Health and should be adhered to strictly This rule can be modified only if sufficient evidence can be accumulated to determine the exact period of infectivity following an attack of viral hepatitis, or it is shown that ultraviolet irradiation of plasma gives certain protection

(2) Recipients of blood or plasma transfusion within the past 6 months should be rejected because of the possibility of transmitting viral hepatitis within that period

(3) Intimate contact with a case of viral hepatitis in the preceding 6 months excludes a donor because of the possibility of transmitting the disease within that period

(4) Persons with a history of healed jaundice due to other causes (neonatorum, gallstones, etc) which no longer exist may be accepted at the discretion of the medical director if he is certain that the jaundice was not of viral origin

(*g*) **Existence of the following conditions** usually disqualifies a donor but the final decision must be made in each individual case by the physician

(1) Tuberculosis Extent of lesion and time elapsed since its occurrence should be considered
(2) Rheumatic fever
(3) Prolonged fevers
(4) Diabetes
(5) Chronic eczema, dermatitis or recurring boils
(6) Cardiovascular disease History and physical findings to be evaluated A history of proven myocardial infarction should exclude the donor
(7) Kidney disease
(8) Persistent cough
(9) Pain in chest
(10) Shortness of breath
(11) Fainting spells
(12) Convulsions
(13) Frequent allergic reactions—hives, food sensitivity, asthma, or wheezing
(14) Active hay fever

(*h*) **Immunizations**

(1) Rabies Donors may be accepted when 2 years have elapsed since last immunization

him, a mouth gag should be inserted and oxygen should be administered, if the physician so directs Hyperventilation tetany may be treated by rebreathing

No donor should leave after recovering from a reaction until authorization has been given by the physician in charge of the operation

Volunteer workers who assist in the care of the donor, under the supervision of the physician and the nurses, should be carefully instructed in the use of ammonia inhalants and warned of the danger of getting ammonia in the eyes Ammonia should be used sparingly, and equipment should be available at all times for adequate eye irrigation

A record of donor reactions should be made on the donor card

Other donor complications—In addition to reactions occasioned by withdrawing blood, there may be local injury such as hematomas, dermatitis from iodine or adhesive, reaction to procaine, or infection at the site of venipuncture There may also be personal injury during syncope, occasioned by falling with resulting abrasions, lacerations, or fractures A medical kit for the treatment of severe reactions and accidents should be on hand at all blood collecting operations It is suggested that the following items of equipment and supplies be available at all times in the recovery room

(*a*) Unsterile—
- Recovery beds or cots
- Blankets
- Shock blocks
- Emesis basin
- Bedpan and urinal
- Small cylinder of oxygen with mask and gauge.
- Mouth gags
- Arm board
- Tourniquet
- Paper towels
- Mouth wipes
- Hand towels.
- Adhesive
- Paper bags

(*b*) Sterile—
- Basic suture set
  - 1 small scissors
  - 1 mouse toothed forceps
  - 1 small needle holder
  - Dermal or silk sutures with atraumatic cutting edge needle.
  - Plain catgut
  - 2 x 2 sponges
  - 2 inch bandage
  - 2 fluted towels for drapes
- *Recipient set*
  - 2 cc syringes with needles
  - 20 cc syringes with needles

Reactions

Although the withdrawal of blood is usually accomplished with no untoward reaction, a small percentage (4-6 percent) of donors show some systemic reaction before, during, or following the venipuncture This is due to a number of causes, not the least of which are fear and apprehension.

Many factors predispose to reaction These may include warm, noisy or crowded donor room, air conditioned donor room with warm canteen, epidemic fainting, fatigue, hunger, youth, history of previous syncope, and too rapid a rate of blood withdrawal Most reactions usually begin with pallor and perspiration This may proceed to a slow, weakened pulse and loss of consciousness There may be nausea, dizziness, tetany, and convulsions Reactions are usually classified as slight, moderate and severe Slight reactions are unaccompanied by syncope, moderate reactions consist of subjective symptoms and fainting, while severe reactions are those in which syncope is accompanied by convulsions or hyperventilation tetany

Prevention—It is much better to prevent a reaction than to treat one All personnel assisting in a blood collection operation should be ever vigilant in observing and forestalling unfavorable donor reactions An attractive, soothing environment, well organized handling of the donor, diversion of the donor s attention during blood collection, an attitude of assurance and sprightly conversation will do much to prevent the onset of reactions Fruit juice should be given prior to donation to elevate the blood sugar A steady, slow rate of blood withdrawal, not exceeding 100 cc per minute, is essential in order to keep the incidence of reactions low Adequate rest following donation is also necessary Tired, hungry, or frightened donors are the most likely candidates for reactions Rest, light refreshments, reassurance are the proper antidotes

Management—The withdrawal of blood from a donor should be discontinued at the first symptom of syncope, and the physician in charge summoned The donor should be reassured, his clothing loosened, and feet raised to higher than head level If he is slow in recovering, he should be removed to a recovery bed which is screened from observation of other donors

If syncope threatens to appear following donation while the donor is eating, the donor's head should be lowered between his knees and ammonia administered as an inhalant. If symptoms progress, he should be lowered to the floor with caution against injury, and the physician should be summoned The donor may then be removed to the recovery room No reacting donor should be left alone at any time When a donor is having convulsions, the first effort should be to protect him from injuring himself Without violently restraining

dropped into a solution of copper sulfate it is encased in a sack of copper proteinate and the density of this discrete drop is unchanged for about 15 seconds. The rise or fall of the drop during this interval indicates whether its specific gravity is greater or less than that of the solution Since Minimum Requirements of the National Institutes of Health states that donors should have a hemoglobin of 12 5 grams percent or more, a copper sulfate solution with this specific gravity is used for screening purposes Copper sulfate with a specific gravity of 1 053 is equivalent to blood containing 12 5 grams percent of hemoglobin

To insure protection of the donor and accuracy of the test, special attention should be paid to the following

(*a*) Equipment

(1) Flasks used for the copper sulfate solution must be thoroughly clean and flushed with the copper sulfate solution before being filled with a known quantity of solution

(2) Individual, sterilized lancets must be used to prevent the transmission of the hepatitis virus Heat sterilization may be accomplished by

a Autoclaving 121 5° C —15 lbs per square inch of saturated steam, 30 minutes

b Boiling in water at 100° C (only after thorough cleansing with soap and water) 30 minutes

c Dry heat 170° C for 2 hours (lancets must be clean)

(3) A new capillary tube should be used for each determination The bulbs may be used repeatedly

(*b*) Solution

(1) The copper sulfate solution, when not in use, should be tightly stoppered to prevent evaporation and possible changes in specific gravity (When the environmental temperature is high it may be necessary to immerse the $CuSO_4$ container in an ice water bath and stopper it between each determination)

(2) One determination can be made per cc of copper sulfate solution However, best results are obtained if not more than 75 percent of the possible determinations are made in any given volume of solution

(3) At least 1 minute should elapse between each determination to allow the solution to clear

(4) Keep the surface of the solution clear and free from fragments

(5) Avoid convection currents which might cause false readings When cold bottles of solutions are brought into a warm room, they should be allowed to reach room temperature before being used

(c) Drugs—
- Ampules
  - Aromatic ammonia
  - Caffeine sodium benzoate
  - Aminophyllin.
  - Epinephrine
  - Coramine
  - Ephedrine
  - Calcium gluconate.
  - Sodium amytal.
  - Atropine
  - Distilled water
- Elixir phenobarbital
- Aspirin
- Glucose 5 percent in saline
- Three units of plasma (or plasma expander)

When the blood bank is not in a hospital, it is suggested that specific arrangements be made with a local hospital for the care of minor injuries or illness so that donors incurring such may be immediately placed under the care of a competent physician in the community

Techniques Used in Connection With the Donor

Donor belt line.—In the interest of efficiency it is essential that donors proceed through the blood collection operation in an orderly "belt line" fashion in the following steps

(a) Registration At this time the donor registration card is initiated

(b) The donor should sign a release form, permitting the withdrawal of his blood

(c) Temperature and weight are measured and recorded

(d) Blood pressure, pulse, and hemoglobin are measured, medical history taken and recorded

(e) Fruit juice is offered to the donor

(f) Donor proceeds to donor room

(g) After appropriate recovery following donation, donor receives refreshments, preferably in a canteen area

Hemoglobin determination.—Selection of donors with an adequate hemoglobin level is probably the most important single criterion in the operation of a successful blood program However, the accurate determination of hemoglobin is a precise laboratory procedure Considerable experience indicates that while not ideal, the most useful and generally reliable method now available for screening donors is the modified copper sulfate technique of Phillips and Van Slyke[1] This method is based on the principle that when plasma or whole blood is

[1] Journal of Biological Chemistry 183 305-360 March 1950

(c) Preparation of donor bottle

(1) Donor bottles containing ACD solution should be precooled to 4° to 10° C, when possible, before being used

(2) *The bottle label, the pilot tube and the two specimen tubes must bear the whole blood number* The pilot tube[*] should always be sterile to prevent the possibility of false reactions due to bacterial contamination when the bleeding is crossmatched at some later date It is the responsibility of the person performing the venipuncture to check each label carefully to be certain that the numbers are identical, to place the whole blood number in the proper space on the donor registration card, and to greet the donor by name to verify that appearing on the card Blood collected into ACD solution for whole blood transfusion must have a pilot tube affixed to the blood collecting bottle, preferably to the left of the label This is easily done by using 1½ turns of waterproof cellophane tape applied near the top of the pilot tube and also near the bottom, if possible However, the tape should not pass over the blood bottle label, which must be left free for required entries The two additional tubes required for laboratory testing (grouping, typing, serology, etc) may be fastened to the bottle by a rubber band

(3) The rubber stopper of the donor bottle should be prepared with 2 percent aqueous iodine and alcohol before the insertion of the bottle puncturing needle of the donor set.

(4) The clamp of the donor set should be securely closed and the bottle puncturing needle inserted through the designated space on the bottle stopper

(5) The bottle should be inverted and action of the anticoagulant solution noted The appearance of an excessive number of bubbles denotes leakage of air, and bottle and set must be discarded

(6) Sides of the bottle should be coated with anticoagulant solution and the bottle placed in an inverted position (in bottle rack if available)

(*d*) Placement of tourniquet

(1) The tourniquet should be placed at a point 2 inches above planned site of venipuncture to aid in selection and fixation of the vein, and then released so that the skin may be "prepped" for venipuncture

(2) If a blood pressure cuff is used as a tourniquet, the pressure should be maintained near 60 mm Hg

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[*] Standard tubes are without anticoagulant. (Clotted blood is preferable for storage for crossmatching)

(c) **Technique**

(1) The finger selected for puncture should be carefully cleansed with alcohol If necessary, finger should be scrubbed with a soapy sponge before applying alcohol

(2) The puncture should be made to the side of the tip of the finger with sufficient force so that an adequate amount of blood is secured without "milking" finger

(3) After wiping off the first drop with a sterile dry sponge, collect sufficient blood in the capillary tube so that there will be a slight excess after a full drop has been expelled

(4) Hold delivery end of the capillary tube one half inch above solution The tube may be supported on the side of the cylinder Apply gentle steady pressure to the bulb and deliver one drop into center of solution surface

(d) **Interpretation**

(1) If the drop of blood is heavier than the test solution and continues to sink during the first 15 seconds after it has entered the solution, the person is acceptable

(2) If the drop is of the same specific gravity as the test solution and becomes stationary during the 15 seconds following its entrance into the solution, the person is acceptable

(3) If the drop is lighter than the test solution and rises, perhaps only a few millimeters, during the first 15 seconds before starting to sink to the bottom, the person is ineligible for donation The test should be repeated if the action of the drop is questionable or unsatisfactory In case of doubt after retesting, it is better to reject a donor who might possibly be made anemic if accepted with a borderline hemoglobin level

Venipuncture—The following are important factors in the performance of venipuncture

(a) **Equipment**

(1) Blood collecting bottle Type 4, glass, 690±15 cc capacity with metal bail attached to bottle by metal band Bottle contains 27 inches of vacuum and 120 cc of ACD solution (NIH solution B, see purchase description supplement B)

(2) Blood collection set 22 inch length of high grade translucent rubber tubing or plastic tubing, fitted at one end with a 17 gage intravenous needle and at the other end with a 15 gage stopper puncturing needle bearing a suitable rigid member securely fastened to the hub to aid in inserting the needle A suitable clamp is necessary

(b) **Selection of vein**

(1) Only the veins in the antecubital fossae of the arms are to be used for the withdrawal of blood As a rule, no more than one puncture should be attempted in each arm

(*c*) **Technique**

(1) The finger selected for puncture should be carefully cleansed with alcohol If necessary, finger should be scrubbed with a soapy sponge before applying alcohol

(2) The puncture should be made to the side of the tip of the finger with sufficient force so that an adequate amount of blood is secured without "milking" finger

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(*b*) **Selection of vein**

(1) Only the veins in the antecubital fossae of the arms are to be used for the withdrawal of blood As a rule, no more than one puncture should be attempted in each arm

of blood should be discontinued and treatment for the reaction should be instituted (See Management under Reactions, p 130)

(5) When the desired quantity of blood has been withdrawn, the clamp of the donor set should be tightly closed and the needle removed from the donor bottle. The required number of specimens should be collected by the person doing the bleeding, the tourniquet should be released and the needle removed from the donor's arm

(6) The bottle of blood should be gently inverted several times and promptly refrigerated.

(7) Every effort should be made to secure a full bottle of blood In collecting blood for other than local use a bottle should contain at least 550 cc. of blood and ACD diluent, because of the distances involved in shipping Bottles containing less than these amounts should not be shipped but may be used locally provided total volume is at least 370 cc Smaller bleedings should not be used for transfusion of children

(8) Bottles that are considered potentially contaminated (PC) should not be shipped for outside use

(*h*) Care of donor following venipuncture

(1) Immediately after the withdrawal of the venipuncture needle, steady pressure should be applied to the puncture site, with the donor s arm elevated, but not flexed Pressure should be maintained for 1 to 2 minutes

(2) A dressing should be securely applied to the venipuncture site and the donor should be instructed to leave the dressing in place for 4 to 6 hours

(3) The donor should rest for 10 to 15 minutes following the withdrawal of blood He should not be left alone during this period

Storage of Blood

Uniform temperature is of the utmost importance for the proper preservation of whole blood Fluctuation of temperature during storage permanently diminishes the survival time of stored red cells and is conducive to fibrin precipitation The damage done by warming blood cannot be compensated for by any amount of subsequent careful refrigeration Therefore, immediately after blood is collected, it must be placed in a refrigerator container capable of maintaining a constant temperature of 4° to 10° C (preferable 4° to 6° C) Blood must not be allowed to freeze

After blood has been placed in a refrigerator, it must not be taken out and kept at room temperature for any appreciable length of time

(3) With either type of tourniquet, a palpable radial pulse indicates that the tourniquet is not too tight

(*e*) **Preparation of venipuncture site** An area 4 x 4 inches should be carefully prepared An acceptable method is as follows

(1) Vigorously scrub skin with a sterile gauze sponge saturated with 50 percent aqueous solution of green soap

(2) Remove soap with sterile applicator saturated with 70 percent alcohol

(3) Apply 2 percent aqueous iodine to site, using sterile applicator Allow iodine to dry

(4) Inject approximately ¼ cc of 1 percent procaine intracutaneously Disposable procaine injection units or individual, sterile syringes and needles should be used

(5) Remove iodine solution with sterile applicator saturated with 70 percent alcohol

(6) Perform the venipuncture

(*f*) **Venipuncture** An acceptable technique is as follows

(1) No manual palpation of the vein should be done in the clean area after the site has been prepared The venipuncture should be accomplished by a non touch technique Anchor the vein by drawing skin tight outside of prepared area

(2) Hold needle at 30°–45° angle with bevel up and pierce skin directly over vein with a sharp thrust Lower angle of needle to 10°–15° and with a slow, steady push enter the vein wall Needle should be inserted into lumen of the vein for a distance of approximately one half inch to anchor it firmly in place

(3) Cover site with sterile sponge and place strip of adhesive across hub of needle With few exceptions, it is not necessary to hold the needle in place with the fingers

(*g*) **Collection of blood**

(1) After venipuncture has been performed, the clamp should be carefully adjusted to permit blood to enter the anticoagulant at a very slow rate Sudden gushing of blood into the bottle must be avoided

(2) Allow 5 to 7 minutes for the withdrawal of 480 cc of blood (to the 600 cc mark—the correct amount for 120 cc ACD solution) A faster rate of collection greatly increases the likelihood of a donor reaction Speed of flow should be adjusted so that blood flows steadily without collapsing the vein

(3) Blood collected into ACD solution should be carefully agitated with a circular, gentle motion at least five times during the withdrawal

(4) The donor should be carefully observed during the entire procedure If he shows symptoms of a reaction, the withdrawal

follow the color system specified in the National Institutes of Health Minimum Requirements for Whole Blood (supp A), which specifies yellow—Group A, pink—Group B, blue—Group O, white—Group AB, and black on white for Rh types

Bottles may be prepared ahead of time, with sterile pilot tube fixed to the bottle with cellophane tape and the laboratory tubes held on by rubber bands The rectangular space at the bottom of the basic label may be covered with a printed (or stamped) gummed strip bearing the blood bank s name and location (fig 5), or this data may be written in The whole blood numbers should be placed on the bottles and on the tubes, just before use, at a table set up for this purpose outside of the donor room The unnumbered bottles are kept in a refrigerator or a refrigerated box and removed when needed as donors present themselves.

Labeling and packing should be done in a wall in refrigerator, if possible If this cannot be done, blood should not be kept outside the refrigerator for more than 15 minutes for labeling

Mechanical refrigerators for the storage of blood should be equipped with a fan to circulate the inside air, an adequate alarm system, and preferably, a device for making a permanent continuous record of the temperature within

During mobile unit collections, an extra insulated refrigerated box should be on hand to minimize opening and closing One box should be used to store bottles of blood as they are collected As soon as six bottles have been accumulated, these six can be placed at one time in an empty 24 unit box already cooled to the desired range This box is then closed and not opened again until the next six bottles are ready to be placed inside In this way, it is possible to keep a more even temperature for most of the blood during its storage at the site of collection

Distribution of Blood

Labeling—Every bottle of blood must be properly identified both as to its contents and the laboratory responsible for its safety Bottles containing ACD anticoagulant solution will be purchased from the manufacturer by FCDA for civil defense reserve supplies, with a basic blood label already applied, as shown in figure 1 Labeling is more easily accomplished by adding to the basic label, locally obtained printed gummed[3] labels showing the whole blood number, the group and type, blood bank identification, and other required data However, blank labels may be used instead with stamped or hand written data, or the required information can be written directly on the appropriate spaces of the basic label

Whole blood numbers (fig 3) may be obtained locally in booklets of printed gummed labels—five labels for each number—one for the bottle, one for the pilot tube, one each for the two laboratory tubes and one for the donor registration card Whole blood numbers may carry a different numerical or letter prefix for each collecting bank or mobile unit, where this is desirable Similarly, the group and type labels (fig 2) may be printed, as well as the blood bank identification (fig 5), the "Proven Group O" and the "High titered, Proven Group O, for Group O Recipients Only" labels, as shown in figure 4 Printed labels must be the same size as shown in each figure to conform to box sizes on the basic label

If printed labels are obtained for designating the blood groups and types, they should all be in black ink on white paper, or they should

[3] Gum should be of a water resistant type for protection during storage and shipping

follow the color system specified in the National Institutes of Health Minimum Requirements for Whole Blood (supp A), which specifies yellow—Group A, pink—Group B, blue—Group O, white—Group AB, and black on white for Rh types

Bottles may be prepared ahead of time, with sterile pilot tube fixed to the bottle with cellophane tape and the laboratory tubes held on by rubber bands The rectangular space at the bottom of the basic label may be covered with a printed (or stamped) gummed strip bearing the blood bank s name and location (fig 5), or this data may be written in The whole blood numbers should be placed on the bottles and on the tubes, just before use, at a table set up for this purpose outside of the donor room The unnumbered bottles are kept in a refrigerator or a refrigerated box and removed when needed as donors present themselves

BASIC WHOLE BLOOD LABEL

FACE

Manufacturer's Name—ACD Lot No

CITRATED WHOLE BLOOD
(HUMAN)

Contains 480 cc of human blood plus 120 cc Anticoagulant Acid Citrate Dextrose Solution B U S P (NIH Solution B)

CAUTION

1. Store continuously at 4 to 10 C (39 to 50 F) preferably at 4 6 (39 to 43 F)
2. CROSSMATCH BEFORE USING
3. Shake vigorously before using
4. ALWAYS ADMINISTER THROUGH A FILTER
5. Administer without warming
6. Read label carefully to assure that blood group and type are the same as those of the recipient
7. Do not add other medication to bottle of blood prior to administration

WHOLE BLOOD NUMBER

BLOOD GROUP TYPE,
EXPIRATION DATE
SEROLOGY BLOOD BANK

Figure I

BASIC WHOLE BLOOD LABEL

REVERSE

The left side of the face of the label is for use after the bottle is filled with whole blood.

The blank spaces on the right side and bottom of the face of the label are for the blood bank's use in completing the label when the bottle is filled with blood.

(Manufacturer's name and data—i e., ACD formula, sterile, non-pyrogenic, etc)

FIGURE 1a

BLOOD BANK LABELS

Group and Type

GROUP O	GROUP A	GROUP B	GROUP AB
Rh POSITIVE Tested for Rh (D)	Rh POSITIVE Tested for Rh (D)	Rh POSITIVE Tested for Rh (D)	Rh POSITIVE Tested for Rh (D)
EXPIRATION DATE	EXPIRATION DATE	EXPIRATION DATE	EXPIRATION DATE
SERUM NEGATIVE TO	SERUM NEGATIVE TO	SERUM NEGATIVE TO	SERUM NEGATIVE TO
Mid County Blood Bank Mid City N Y License No 000	Mid County Blood Bank Mid City N Y License No 000	Mid County Blood Bank Mid City N Y License No 000	Mid County Blood Bank Mid City N Y License No 000

GROUP O	GROUP A	GROUP B	GROUP AB
Rh NEGATIVE Tested for Rh (D)	Rh NEGATIVE Tested for Rh (D)	Rh NEGATIVE Tested for Rh (D)	Rh NEGATIVE Tested for Rh (D)
EXPIRATION DATE	EXPIRATION DATE	EXPIRATION DATE	EXPIRATION DATE
SERUM NEGATIVE TO	SERUM NEGATIVE TO	SERUM NEGATIVE TO	SERUM NEGATIVE TO
Mid County Blood Bank Mid City N Y License No 000	Mid County Blood Bank Mid City N Y License No 000	Mid County Blood Bank Mid City N Y License No 000	Mid County Blood Bank Mid City N Y License No 000

FIGURE 2

Instruction —These label one for each blood group—Rh positive and Rh negative bearing the expiration date erology (name of test) and the blood bank identification are affixed as appropriate in the lower right hand pace of the basic blood label

When printed or blank stick on labels are not available all but the blood bank identification can be written or stamped in the blank area of the basic label If more pace is required the blood bank identification may be written or stamped in the lower left hand pace of the basic blood label.

BLOOD BANK LABELS

Whole Blood Number

WHOLE BLOOD NUMBER 543210 MID CITY, N Y	WHOLE BLOOD NUMBER 543210 MID CITY, N Y	WHOLE BLOOD NUMBER 543210 MID CITY, N Y	WHOLE BLOOD NUMBER 543210 MID CITY, N Y	WHOLE BLOOD NUMBER 543210 MID CITY, N Y

FIGURE 3

Instruction —These labels should be obtained in perforated strips of five identical numbers. One is placed on the donor card one in the upper right hand space of the basic blood label one on the pilot tube and one on each of two specimen tubes for laboratory tests

Blank labels may of course be used and the appropriate number entered or it may be written or stamped directly in the space provided on the basic blood label.

Special Group O Labels

PROVEN O

MAY BE USED WITHOUT CROSSMATCHING

HIGH TITERED

FOR GROUP O RECIPIENTS ONLY

PROVEN GROUP O

FIGURE 4.

Instruction —These labels are to be applied when appropriate in the lower left hand space of the basic blood label. Blank labels may also be used or the appropriate statement entered directly on the space provided on the basic label

gives the day and hour that the ice compartment was last filled Transportation agents should be instructed that the seal on boxes of blood is not to be broken except in time of emergency when it is evident that more than 24 hours will elapse from the time the boxes were last filled with ice before they can be delivered to their destination Under these circumstances, the shippers must arrange to have the seal broken, the ice compartment refilled and a note describing the circumstances and time of breaking the seal added to the tag tied to the handle of the box All previous shipping labels should be removed from a refrigerated container each time it is released to the shipper The presence of multiple address labels on the box can easily lead to mistakes in the delivery of blood Since time is of the utmost importance in the shipment of blood, everything possible must be done to avoid delay en route The civil defense authority to whom the shipment of blood is consigned should be informed by means of official civil defense communication (telephone, wire, radio) of the number of bottles, the time of departure, and the flight, train, or truck identification number of each shipment of blood

Laboratory Techniques

Determination of blood groups—Although there are two satisfactory standard techniques in general use, the slide method and the test tube method, only the former is recommended for use in civil defense emergencies The accuracy of the slide method, its simplicity and economy of equipment and personnel make it best suited for emergency operations All blood banks should become familiar with the slide method and should have proper grouping and typing sera available as part of local reserves for civil defense emergencies Federal reserves of sera will be suited for the slide method Only test sera which are distributed by laboratories under license by the National Institutes of Health should be employed

The slide test method is described below

(*a*) Materials
(1) Clean glass slides or plates
(2) New applicator sticks
(3) Anti A and anti B grouping serum
(4) Whole blood (or a cell suspension from oxalated blood or blood clot sample in 0 85 percent sodium chloride solution)
(5) Illuminated ground glass plate

The glass slides or plates should be marked so as to make parallel spaces each approximately 1 inch square It is *inadvisable* to do tests on more than 10 specimens at one time

(b) **Procedure** A whole blood suspension is simpler to use if care is taken in the amount employed A technique has recently been recommended for rapid typing using whole blood from a finger puncture[8] If a cell suspension is to be used, a cell suspension of not less than 5 percent nor more than 10 percent in saline solution should be made from the clotted blood sample or the oxalated blood sample Weak subgroups easily detected with whole blood may be missed when too light a cell suspension is used

(c) **Test.**

(1) Place one drop of the whole blood (or 10 percent cell suspension) in each of two squares

(2) Place a drop of anti A group serum on the left side square, and a drop of anti B group serum on the right side square

(3) Mix the cells and serum with the end of an applicator stick with *due caution* that each square is treated as a separate test and a different stick end is used in each square

(4) Rotate or incline the slide to keep the mixture moving

(5) Observe for agglutination over an illuminated ground glass plate A and B hemoglutinins are weakened in their action by heat and agglutination may even be reversed Thus technicians should use care to avoid any appreciable heating of the slide preparation

(d) **Interpretation**

Where indicated, agglutation should appear within a few seconds in the form of distinct clumps The clumps should be 1 sq mm in surface area following three minutes of continuous rotation

No agglutination on either side indicates group O cells

Agglutination on the anti A serum side indicates only group A cells

Agglutination on the anti B serum side indicates only group B cells

Agglutination on both the anti A and anti B sides indicates group AB cells

Group confirmation—The importance of correct results demands that the blood group be determined by a second method

As indicated in paragraph 4 18 (c), page 14, this is preferably done for group O bloods by testing the cells a second time against high titered O serum (anti A and B), since time will probably not permit the use of the more commonly accepted method of testing the donor's serum with known A and B cells.

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[8] *An Accurate Method for Rapid Blood Grouping of Large Numbers of People* Fred H Allen Jr Louis K Diamond and Helen J Madden N E J Med, 244 925 (June 21) 1951

which equipment and standardized reagents are available for daily needs Any tests described in the *Manual of Serologic Tests for Syphilis Supplement No 22, The Journal of Venereal Disease Information*, 1949, published by the United States Government Printing Office, Washington 25, D C, and procurable for $1 50 a copy, may be used The responsible technician should have had specific instruction and experience in the method to be used

Isoagglutinin titer—Determination of the agglutinin titer of group O blood is discussed in par 4 18 (f), p 17

EMERGENCY BLOOD TRANSFUSION

[Federal Civil Defense Administration, Technical Bulletin, TB-11-5, November, 1952]

This supplement recommends minimal procedures for blood transfusion in civil defense emergencies It describes procedures for the initial emergency or *O-P phase* when cross matching will be impossible and only group O blood, plasma, or plasma volume expanders can be relied upon, the *transitional phase* when group specific transfusions, employing emergency techniques, will begin, and the *recovery phase* when standard techniques for group specific transfusions will be re-established These minimal procedures are not intended to be used when conditions permit the proper handling of blood Improper handling of blood for transfusion leaves no margin of safety

Material in this supplement was prepared under the auspices of the Committee on Blood, Division of Medical Sciences, National Research Council

Ideal Conditions for Blood Transfusion

Blood transfusion is reasonably safe under ideal conditions These include proper collection, processing, storage, distribution, cross matching, and administration of blood All phases in the handling of blood should be carried out under aseptic conditions, and approved techniques are required throughout All donors should be medically screened to assure their safety and to exclude potentially infectious blood All sera used in typing and serologic tests should be standard with regard to potency and specificity Careful identification of donors, blood bottles, and recipients can only be accomplished through accurate labeling and recording This is especially important during crossmatching

O-P Phase

The initial emergency period following a major disaster is called the O-P phase to emphasize that only group O blood and plasma (or plasma volume expanders) can be relied upon during the first hours Blood of group O can be administered without prior crossmatch to any person requiring an emergency transfusion This must be

accepted and planned for, since facilities for grouping, Rh typing, and crossmatching may be lacking for 12 to 24 hours, or possibly longer Even if certain nearby hospitals are relatively undamaged, available technicians will have more than they can do to meet the immediate demand for group O blood *Group specific transfusions must not be given on the basis of blood typing records from identification cards, tags, or marks without careful crossmatching* Therefore, plasma and group O blood are to be used until laboratory services can provide properly grouped, Rh typed, and crossmatched blood for group specific transfusions

In the O–P phase, casualties requiring whole blood will be those with exsanguinating injuries, especially those who need major surgery for resuscitation, these will be in the minority *Group O blood must be given without regard to blood group or Rh type of the recipient in the initial emergency period*

If group O, Rh negative blood is available without facilities for Rh typing of recipients, it should be reserved for those with exsanguinating injuries in the following priority

(a) Young women and female children.

(b) Women who have had children.

(c) Persons who have had multiple blood transfusions

If group O, Rh negative blood is available, and Rh typing of recipients can be done, Rh negative blood should be given to Rh negative patients This will prevent Rh sensitization.

If low titered group O blood is available, it should be given preferentially to persons who will require many transfusions, since large amounts of high titered group O blood may cause destruction of the recipient's own red cells. (If available, A and B blood group substances should be added to group O blood with undetermined titer.)

Only group O blood can be given to group O recipients, since bloods of other groups are not compatible High titered group O blood when so identified, should be given preferentially to group O recipients

Plasma and plasma volume expanders are indicated in emergency treatment of shock due to hemorrhage, trauma, burns, and similar conditions, when whole blood is either not available or available only in limited amounts Under such circumstances whole blood should be preserved for those cases where hemorrhage has been profound and resuscitation can be accomplished only by replacement of red blood cells In all cases of shock due to severe hemorrhage or burns, plasma and plasma volume expanders should be given only in amounts necessary to meet the acute emergency of shock, and whole blood therapy should be instituted as soon as possible to correct the complicating anemia The majority of the injured will probably not

suffer severe blood loss and therefore will not require immediate or subsequent whole blood transfusion Plasma and plasma volume expanders have the advantage of immediate use without prior grouping or crossmatching and will not cause Rh sensitization

Transitional Phase

A transitional phase in transfusion operations will follow the O-P phase, beginning perhaps in 12 to 24 hours, and possibly continuing for 48 hours Some group O blood will still be used, but the use of group specific transfusions employing emergency procedures will begin during this phase

Recovery Phase

As soon as possible, standard accepted techniques for group specific transfusion should be reestablished The slide test methods for ABO blood grouping, Rh typing, and crossmatching are described in appendix A. The test tube crossmatch technique should always supplant the slide technique as soon as facilities permit.

Group-Specific Transfusions

Only blood of group O can be given without crossmatch, and this is strictly an emergency procedure Transfusions of unmatched group O blood should be abandoned as soon as conditions permit to conserve blood for group O recipients Donor blood of the other groups must always be crossmatched with blood from the recipient before administration

Donor blood of the recipient's blood group should be selected and the two should be carefully crossmatched with a technique which will reveal both blood group and Rh incompatibilities Although Rh negative blood can be given safely to Rh positive persons, the supply will be limited and should be reserved for Rh negative patients

During the transitional phase, and when limited blood grouping and matching facilities are becoming available, it may be desirable as a first step to expand collection of blood to include group A in addition to group O donors Group O blood can then be crossmatched for group O and group B recipients, and group A blood for group A and AB recipients It is important that the major side of the cross match (donor cells and recipient serum) be double checked for compatibility when cells of different groups are matched

Processing Blood Outside a Disaster Area

All facilities surrounding a disaster area should be alerted immediately following an attack They should collect and process all group

O blood available from the population, indicating Rh positive or negative Blood which is not group O may also be collected, processed, and stored for use in the recovery phase or for conversion to plasma or its derivatives (groups A and B are ideal for plasma processing)

Blood collecting stations surrounding the disaster area and in other parts of the country must observe accepted minimum standards set forth in Supplement C, Standard Operating Procedures for the Collection and Processing of Blood for selection of donors, collection, processing, and storage of blood, using standard equipment

Emergency Processing of Blood In or Near a Disaster Area

During an emergency it may be necessary, in and near the disaster area, to compromise the accepted methods for processing blood under ideal conditions

Selection of Donors

Usual standards for selection of donors may have to be lowered temporarily as follows

(*a*) History of past disease (syphilis, malaria, or viral hepatitis) in a donor may be temporarily ignored except in cases where the disease, even though mild is still active

(*b*) Usual age and minimum weight limits in donors may be extended to include all apparently healthy persons willing to give blood The minimum hemoglobin standard of 12 5 gm should be observed, if possible

(*c*) Usual medical restrictions concerning such chronic disease states as hypertension and diabetes may be relaxed if the removal of 1 pint of blood is not medically contraindicated to the donor The usual donor release form should be obtained, if possible

Determination of Donor's Blood Group

It will be necessary to select group O donors for immediate use This must be done with skill, employing known potent reagents, and with accurate identification of the donor with the specimen of blood tested A record card must be kept showing the donor's name, address, and results of examinations made prior to bleeding At the same time the hemoglobin test is done, blood should also be taken from the donor s finger to perform a blood grouping test, preferably on the donor card, as a permanent record of the blood group

The grouping test is performed on the surface of the card (or slide) by mixing a drop of antiserum with a small drop of blood, one quarter the volume of the serum Anti A serum is placed in one space marked "anti A," and anti B serum in a separate space marked "anti

B ' A small drop of blood from the donor s finger is mixed with the anti A serum and spread out over an area about 1 inch in diameter (The finger must not touch the serum on the card) Another drop of blood is mixed with the anti B serum and spread similarly The card is then rocked back and forth and observed for agglutination

If neither of the serum blood mixtures shows agglutination, the person is blood group O If both serum blood mixtures are agglutinated, he is group AB If only the anti A serum mixed with blood produces agglutination, the donor is group A, and if only the anti B serum blood mixture is agglutinated, he is group B (A third space may be provided on the card for Rh_o typing when done as a predonation procedure, or this may be done on a slide with finger blood)

Group O donors can be selected quickly Each donor s card, after drying, supplies a permanent record not subject to clerical error or misidentification, provided the donor s name is checked at the time his blood is mixed on the card, and again when blood is drawn In addition, while the needle is still in the donor's vein during the withdrawal of blood, a sample must be collected into a sterile pilot tube without anticoagulant The tube should be firmly attached to the bottle of blood and used in the laboratory for proving the donor's blood group and for Rh typing

Minimum laboratory procedures for the typing of blood are de scribed in appendix A The slide test method will make it possible to rapidly select group O donors when the record card, described above, is not available In addition, this method will reveal specific blood groups as well as Rh types with the least time and effort during the transitional and recovery phases

To prepare group O blood for emergency use without crossmatching, the blood grouping must be proved by a second procedure This is most easily done by using anti A plus anti B (group O) serum as described in appendix A

IDENTIFICATION OF DONOR BLOOD

The label on donor blood must show an identifying name or number, name and address of the establishment collecting blood, expiration date, and the blood group and Rh type of blood contained in the bottle If the manufacturer's label is the only one available, the above information must be clearly written on it in pencil or waterproof ink (Indelible pencil should not be used because under refrigeration the pencil markings will smear and become illegible)

Crossmatching In or Near a Disaster Area

Advantage should be taken of techniques which will disclose danger ous blocking antibodies as well as the usual agglutinating antibodies This is particularly true of recipients who may be immunized to Rh

antibodies, such as women who have had children and persons who have had previous transfusions In performing crossmatching tests, both donor and recipient sera should be inactivated by heating for 10 to 15 minutes in a water bath at 55°–56° C If this is not done, hemolysis may occur, producing a false negative report

Both sides of the crossmatch should be set up as a routine, since this takes little longer than setting up only the major side Incompatibility on the major side indicates that the blood must not be given, since a very serious or possibly fatal reaction would result Minor side incompatibilities, although indicating failure of complete group and type matching, do not usually cause serious reactions and may be ignored in extreme emergencies Techniques for crossmatching are outlined in detail in appendix A

Administration of Blood

A sterile pyrogen free administration set with 70 to 100 mesh in line filter should be used Before administration the blood must be thoroughly mixed to produce a uniform suspension Refrigerated blood should not be warmed prior to or during administration, or allowed to stand at room temperature longer than 1 hour before use Heat may injure the red cells and produce excessive hemolysis when transfused The rapid administration of cold blood should produce no adverse effect

Mixing blood with medications or solutions which are likely to produce hemolysis or cause a precipitation of plasma proteins should be avoided Mixing within the tubing of the administration set with other than isotonic solutions is likely to produce hemolysis Medications should not be injected into the tubing carrying blood Administration sets should be rinsed with physiological saline if they have been used to give other solutions prior to the administration of blood Similarly, after a transfusion of blood, the filter and tubing should be cleaned by running 50 to 100 cc of saline through them before other fluids are given If saline is not available, or is contraindicated, 5 percent dextrose in water should be used

One of the most frequent and dangerous errors in crossmatched group specific transfusions is the administration of blood to the wrong recipient This must be prevented by careful identification of recipients, making certain that each gets the blood crossmatched for him Special precautions are required for persons with common names The emergency medical tag serial number described in *Organization and Operation of Civil Defense Casualty Services*, Part III—*Medical Records for Casualties*, TM–11–3, should be used and identification confirmed by full name if possible, preferably by a third person, before starting the transfusion.

Complications of Blood Transfusions

HEMOLYTIC REACTIONS

Clinically, these potentially fatal reactions to whole blood transfusion are characterized by severe lumbar pain, sense of substernal oppression, violent chills, fever, and often rapid, labored breathing, prostration, and hypotension. The severity and time of onset vary, symptoms usually appear after 50 cc or more of blood have been given. In Rh reactions the onset may be delayed for 1 to 2 hours after the start of the transfusion. Jaundice and renal insufficiency may occur later. The mortality rate of hemolytic reactions is relatively high in patients who develop renal failure with anuria. However, those who recover usually have no residual kidney damage.

The pathogenesis of the transfusion reaction stems from the antigen antibody reaction which occurs with the transfusion of incompatible red cells, resulting in excessive hemolysis in the recipient.

Reactions having severest consequence are due to transfusion of ABO or Rh incompatible blood. Hemolytic reactions of lesser significance may also be due to the administration of compatible blood which has been improperly stored, e. g., poor refrigeration.

Reactions may also result from improper administration of blood. This may occur with excessive heating or the improper mixing of solutions with the blood.

Reactions to Rh incompatible blood depend upon acquired isosensitivity and occur most frequently in Rh negative persons. Sensitization develops during pregnancy in about 5 percent of Rh negative women bearing Rh positive children and occurs after repeated transfusions in about 5 to 30 percent of Rh negative recipients. In general, at least 1 to 3 Rh incompatible transfusions are necessary before significant sensitivity develops. Antibodies appear in 1 to 2 weeks.

The incidence of hemolytic reactions is not accurately known, but they occur chiefly because of human error.

Treatment of hemolytic reaction consists of the following:

(*a*) Stop transfusion at the earliest symptom.

(*b*) Treat shock, if present, by the usual methods for supporting the circulation with plasma, plasma volume expanders, or known compatible blood if available. (Recheck typing and crossmatch to locate and rectify error.)

(*c*) Give small amounts of barbiturates to allay anxiety, or morphine if the pain is severe.

(*d*) Administer sufficient amounts of appropriate fluids parenterally to maintain adequate urine output and proper hydration. Fluid and electrolyte therapy is of great importance, especially if renal failure with anuria or oliguria develops.

antibodies, such as women who have had children and persons who have had previous transfusions In performing crossmatching tests, both donor and recipient sera should be inactivated by heating for 10 to 15 minutes in a water bath at 55°-56° C If this is not done, hemolysis may occur, producing a false negative report

Both sides of the crossmatch should be set up as a routine, since this takes little longer than setting up only the major side Incompatibility on the major side indicates that the blood must not be given, since a very serious or possibly fatal reaction would result Minor side incompatibilities, although indicating failure of complete group and type matching, do not usually cause serious reactions and may be ignored in extreme emergencies Techniques for crossmatching are outlined in detail in appendix A

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Mixing blood with medications or solutions which are likely to produce hemolysis or cause a precipitation of plasma proteins should be avoided Mixing within the tubing of the administration set with other than isotonic solutions is likely to produce hemolysis Medications should not be injected into the tubing carrying blood Administration sets should be rinsed with physiological saline if they have been used to give other solutions prior to the administration of blood Similarly, after a transfusion of blood, the filter and tubing should be cleaned by running 50 to 100 cc of saline through them before other fluids are given. If saline is not available, or is contraindicated, 5 percent dextrose in water should be used

One of the most frequent and dangerous errors in crossmatched group specific transfusions is the administration of blood to the wrong recipient This must be prevented by careful identification of recipients, making certain that each gets the blood crossmatched for him Special precautions are required for persons with common names The emergency medical tag serial number described in *Organization and Operation of Civil Defense Casualty Services*, Part III—*Medical Records for Casualties*, TM-11-3, should be used and identification confirmed by full name if possible, preferably by a third person, before starting the transfusion.

Treatment should be prompt and the transfusion stopped with the onset of symptoms and signs of circulatory embarrassment. Oxygen should be administered if available. Tourniquets applied to all four extremities (to stop venous but not arterial flow) will give relief in a large number of patients. In some cases phlebotomy will be necessary.

Prevention of overloading the circulation is contingent on the medical evaluation of each clinical situation. The rate of infusion should not exceed 5 cc. per minute at the start of the transfusion, except in the presence of shock.

PYROGENIC REACTIONS

Clinically, pyrogenic reactions are the most common. They are characterized by chills and fever occurring during or within 1 hour after transfusion and usually lasting less than 4 hours. They may be mild or very severe with violent chills and fever of 104° or 105° F. Recovery is generally complete within 24 hours. If there is difficulty in distinguishing between a pyrogenic and a hemolytic reaction, the finding of an abnormally high amount of bilirubin in the serum will be conclusive evidence of a hemolytic reaction.

Treatment is symptomatic along with prompt discontinuance of the transfusion if the reaction is severe.

CONTAMINATED BLOOD

Grossly contaminated blood must not be given under any circumstances. If inadvertently given, the patient will show symptoms and signs of being severely and acutely ill with a bacteremia. Treatment should be prompt, with large doses of available antibiotics, adequate treatment to combat shock if present, and the necessary symptomatic care.

If possible, the bottle of blood should be returned to the laboratory for culturing and identification of the specific organism so the patient can be treated with appropriate antibiotics.

ALLERGIC REACTIONS

Clinically, an allergic reaction is common and usually characterized by urticaria and occasionally by angioneurotic edema, severe or giant urticaria, and asthma. The urticaria, generally localized, appears during or soon after transfusion. Patients with severe reactions develop asthma or angioneurotic edema, the most dangerous variety involving the glottis. Duration rarely exceeds a few hours.

Treatment is entirely symptomatic. In mild cases 0.3 to 0.5 cc. of 1:1,000 solution of epinephrine can be given subcutaneously. This dose may be repeated at intervals of 20 minutes to 1 hour. In severe cases 0.5 cc. intramuscularly or 0.3 cc. intravenously may be needed. Antihistaminic drugs may be helpful. Usually the transfusion can be continued in the milder urticarial reactions, but if asthma or angioneurotic edema appears it should be stopped immediately.

(*e*) During the acute phase of the reaction only a few persons will be able to take fluid by mouth The insensible loss will approximate 1,000 cc per 24 hours This water loss plus all measurable fluid losses should be conscientiously replaced each day During the period of oliguria or anuria, little or no sodium will be excreted in the urine, therefore, salt intake should be carefully restricted However, if the transfusion reaction occurs in an already dehydrated and salt depleted patient, the judicious administration of salt and water may in itself be necessary for the maximum function of the injured kidney Once these needs have been met, careful daily replacement of water (dextrose in water) is essential together with repeated reevaluation of the electrolyte balance of the patient

(*f*) Alkalinization of the urine should not be attempted, since the relatively large amount of sodium administered as lactate may be retained and may precipitate pulmonary edema An alkaline solution should be used cautiously only during later stages of renal shutdown, to correct acid base imbalance

(*g*) As long as renal insufficiency persists, there is a danger of death from pulmonary edema with the excessive administration of saline The great majority of patients who experience transfusion reactions will survive with good renal excretory function Despite severe oliguria, conservative treatment in most cases will be rewarded by recovery within 2 or 3 weeks It is recommended that 100 to 200 gm of carbohydrate per day be given the patient This is a basic metabolic requirement and will tend to spare protein stores of the body

Careful typing and crossmatching of both donor and recipient and accurate recordings of these data for proper identification of each bottle of blood during handling, processing, and administration is obviously required for prevention of hemolytic transfusion reaction Proper hydration of the patient being transfused is of great clinical importance The patient who experiences a transfusion reaction and who has had adequate fluids and a good urine flow will probably suffer less severe renal damage and make a more prompt clinical improvement than the patient who is dehydrated and salt depleted when transfused

Circulatory Overload

Clinically, circulatory overload can be a serious complication and is characterized by pulmonary congestion and edema The onset of symptoms may occur during or shortly after transfusion with sudden dyspnea, orthoponea, cyanosis, cough, and physical signs of pulmonary edema These are the symptoms and signs which accompany an excessive increase in blood volume due to transfusion of any solution at too rapid a rate or in too great an amount Older people and those with heart disease are especially susceptible, and caution should be exercised with these persons

APPENDIX A

Emergency Blood Grouping Laboratory Techniques

[Federal Civil Defense Administration, *Technical Bulletin*, TB-11-6, November, 1952]

Blood Grouping and Typing

The slide test method is recommended for determination of blood groups and Rh type cells When medical facilities are limited, this technique is considered superior because of its accuracy, simplicity, and economy of equipment and personnel

ABO Grouping (Slide Test Method)

Materials

(*a*) Clean glass slides or plates

(*b*) New applicator sticks and medicine droppers

(*c*) Illuminated ground glass plate

(*d*) Anti A and anti B grouping serum, National Institutes of Health (NIH) licensed, or equivalent

(*e*) Clotted blood sample

Method

(*a*) Mark 1-inch squares on the glass slides or plates

(*b*) After stirring the clotted blood with a clean applicator, place a small drop of the free cell suspension on each of two squares

(*c*) On the left hand square place a large drop of anti A grouping serum and on the right hand square place a large drop of anti B grouping serum (The drop of blood should be one quarter the size of the drop of serum used)

(*d*) Mix cells and serum with a clean applicator stick end for each square

(*e*) Rotate or incline the slide to keep mixture moving

(*f*) Observe for agglutination over an illuminated ground glass plate *The mixture must not be warmed* as this may minimize or even reverse agglutination

Interpretation

When agglutination occurs, distinct clumps should appear within a few seconds Within 3 minutes, with continuous rotation, the clumps should be 1 mm in diameter

(*a*) No agglutination on either side indicates group O cells

(*b*) Agglutination with anti A serum *only* indicates group A cells

(*c*) Agglutination with anti B serum only indicates group B cells

(*d*) Agglutination with anti A and anti B sera indicates group AB cells

TRANSMISSION OF DISEASE

The practice of accepting as donors only those who state they are in good health, and who, on examination, show no apparent evidence of active illness, eliminates most of the disease transmission hazard in blood transfusion There are, however, three known instances in which these criteria are not adequate

(*a*) Syphilis, during the spirochetemic stage, especially before the serology has become positive or the chancre noted, may be accidentally transmitted to a recipient Penicillin therapy should of course be given if this occurs Prevention is partially accomplished by careful screening of donors and may be assured by refrigeration of blood collections for at least 72 hours before use since the spirochete survives less than 72 hours in blood stored at 0° to 10° C

(*b*) Malaria is transmissible by blood transfusion Plasmodia may appear in the blood stream years after an attack of the disease without producing symptoms In addition, plasmodia may not be detectable by blood smear examination because of their insufficient number Therapy with chloroquine, quinacrine, or quinine will cure malaria transmitted by transfusion Prevention of malaria in recipients lies entirely in the rejection of donors with a history of malaria or those who have had intensive suppressive therapy without clinical manifestations The malaria parasite survives in stored blood as long as the red cells, and is not destroyed by refrigeration.

(*c*) Hepatitis can be transmitted by blood or plasma from donors who are asymptomatic carriers or are in the prodromal stage of the disease The viruses of both infectious hepatitis and of homologous serum hepatitis are transmissible by transfusion Incubation periods vary from 1 to 6 months At present, prevention can only be partially effective by rejecting donors with jaundice or known liver disease, or a history of either type of viral hepatitis, and donors who, within a 6 month period, have had blood or plasma transfusions, or have been intimately exposed to a case of infectious hepatitis

TETANY

The sodium citrate used as anticoagulant may rarely produce tetany following rapid transfusion of large amounts of blood and plasma, especially to infants Therapy consists of intravenous injection of calcium gluconate to restore a normal concentration of free calcium ions in the circulation.

AIR EMBOLISM

Air embolism sufficient to cause fatality is rare It is most likely to occur when blood is administered with an air pressure apparatus rather than by gravity When blood is administered under pressure, a responsible attendant must be present constantly

tion date It must show clearly the blood group and Rh type of blood contained in the bottle If the manufacturer's label is the only one available, the above information must be clearly written on it in pencil or waterproof ink

OPTIONAL PROCEDURES

Additional procedures which should be followed if possible are described below These are not essential for emergency use of blood in a disaster

Determination of Isohemagglutinin Titer in Group O Blood

(*a*) Dilute group O serum 1 200 by adding 0 1 cc of serum to 20 cc of normal saline Label two tubes A and B, and place 0 1 cc of the diluted serum in each

(*b*) Select a group A person, whose cells react strongly with anti-A serum, and a group B person Prepare two tubes, one containing packed A and the other packed B cells Dilute the known group A and group B cells with normal saline to make 2 percent suspensions

(*c*) To the tube marked 'A,' add 0 1 cc of the 2 percent suspension of group A cells To the tube marked 'B,' add 0 1 cc of the 2 percent group B cell suspension Shake both tubes well and centrifuge for 1 minute at 2,000 rpm, or 2 minutes at 1,000 rpm Agitate the tubes gently to dislodge the cell packs, and observe for agglutination If there is no agglutination, the donor blood is labeled "low titered" group O blood If agglutination occurs, the donor blood must be designated 'high titered' group O blood

Rapid Simple Serological Test for Syphilis

Serological testing for syphilis will require recognized quick slide-test procedures rather than the more complicated methods

CROSSMATCHING

Two methods of cross matching blood are recommended The test-tube crossmatch if a centrifuge is available, and the slide test cross match which should be used only if a centrifuge is not available

TEST TUBE CROSSMATCH

Materials

(*a*) Clean serologic test tubes.
(*b*) New applicator sticks and medicine droppers.
(*c*) Physiological saline solution
(*d*) 30 percent bovine albumin solution
(*e*) Water bath, heat controlled, 55°–56° C
(*f*) Water bath, heat controlled, 37° C
(*g*) Centrifuge
(*h*) Clotted whole blood samples from donor and recipient.

PROVING GROUP O

To prepare group O blood for emergency use without crossmatching, the blood grouping must be proved by a second procedure, also using the slide test method. Mix donor blood with licensed (or (equivalent) anti A plus anti B (group O) serum meeting NIH minimum requirements, as in the grouping test with anti A and anti B sera described in the previous section Group O blood must be negative with all three sera

If anti A plus anti B (group O) serum is not available for proving the blood group, the ABO grouping test should be repeated, either by a second technician employing the same sera used in the first test, or by the same technician using anti A and anti B sera of different lot numbers Results of the two independent tests must agree

RH TYPING

Materials

(a) Clean glass slides or plates

(b) New applicator sticks and medicine droppers

(c) Illuminated viewing box, heat controlled at 45°-50° C (If not available, improvise with 60 or 75 watt frosted light bulb to supply light and heat)

(d) Anti Rh_o (anti D) typing serum containing blocking anti bodies for slide test, licensed by NIH, or equivalent

(e) Clotted blood sample

Method

(a) Place one drop of anti Rh serum on a slide

(b) Stir clotted blood sample with an applicator stick, making an approximately 40 percent suspension of cells in the supernatant serum, and add two drops, each equal to the volume of anti Rh serum used

(c) Mix antiserum and cells vigorously with a clean stick and spread the mixture over most of the area of the glass slide

(d) The slide must be placed on a warm (45°-50° C) lighted sur *face to speed the reaction and permit observation*

(e) Tilt the slide back and forth while keeping it warm Observe for agglutination

Interpretation

If agglutination occurs, the blood is Rh positive Blood should not be called Rh negative unless careful inspection at the end of 2 minutes reveals no agglutination

LABELING DONOR BLOOD

The label on donor blood must show an identifying name or number, name and address of establishment collecting the blood, and expira

bodies is enhanced by heat (45°–50° C) Therefore, two separate slide test crossmatch tests are required, one for blood group compatibility and one for Rh Hr compatibility In both tests major and minor sides are set up

Materials

(*a*) Clean glass slides or plates
(*b*) Clean serological test tubes
(*c*) New applicator sticks and medicine droppers
(*d*) 30 percent bovine albumin solution
(*e*) Water bath, heat controlled, 55°–56° C
(*f*) Illuminated ground glass plate for ABO compatibility test.
(*g*) Illuminated viewing box, heat controlled at 45°–50° C, for Rh compatibility test
(*h*) Clotted whole blood samples from donor and recipient

Test I Blood Group Compatibility Test

(*a*) Place small specimens of donor s and recipient s sera in separate clearly marked test tubes and inactivate for 10 to 15 minutes in a water bath at 55°–56° C

(*b*) Major side Place 1 drop of inactivated recipient's serum on a slide and near it a small drop (about one quarter the volume of the recipient's serum) of cells from donor s clotted blood sample Mix cells and serum and spread over central half of slide

(*c*) Minor side Place 1 drop of inactivated donor s serum and a small drop of cells from recipient's clotted blood sample on a second slide Mix as for major side

(*d*) Do not place slides on heated surface Hold over a light source and observe at room temperature for 3 minutes while rocking the slides to agitate the serum cell mixtures

(*e*) If clumping occurs, add 1 or 2 drops of saline to rule out rouleaux If clumping persists, true agglutination is present

Interpretation

If agglutination appears *on the major side, the blood must not* be given Minor side incompatibility will be present when group O blood is matched with recipient blood of other groups or when blood of groups A or B is matched with AB recipients This incompatibility may be ignored in extreme emergencies Otherwise, when any incompatibility is found, groupings should be rechecked If an error is found, new donor blood of the recipient's confirmed blood group should be selected for crossmatching If the blood groups are compatible, the Rh compatibility test may explain the incompatibility

Test II Rh Compatibility Test

Rh antibodies are enhanced by heat Also, most Rh antibodies of clinical significance are of the blocking variety, and therefore are more

Method

(*a*) With medicine dropper remove small specimens of superna tant serum from donor's and recipient's clotted blood samples Place each specimen in a separate clearly marked tube and inactivate for 10 to 15 minutes in a water bath at 55°–56° C

(*b*) Major side Prepare 2 percent suspension in physiological saline of cells from donor s clotted blood sample Into a clean test tube place 1 drop of 2 percent suspension of donor s cells, 2 drops of inactivated recipient's serum, and 3 drops of 30 percent bovine albumin

(*c*) Minor side Prepare 2 percent suspension in saline of cells from recipient's clotted blood sample Into a clean test tube place 1 drop of 2 percent suspension of recipient s cells, 2 drops of inactivated donor's serum, and 3 drops of 30 percent bovine albumin

(*d*) Shake the tubes well and incubate at 37° C for 10 minutes

(*e*) *Centrifuge to pack cells firmly* (2,000 *rpm for* 2 *minutes in* a small radius centrifuge head, lower speeds may be optimal in larger radius heads)

(*f*) Gently agitate the tubes to dislodge cell packs Observe for agglutination

Interpretation

(*a*) Incompatibility in this type of crossmatch may indicate either blood group or other antigen antibody reaction The test detects Rh incompatibilities which will be missed unless a high protein method is used

(*b*) Incompatibility on the major side indicates the blood must not be given, since a serious reaction would result

(*c*) Incompatibility on the minor side is present when group O blood is matched with recipient blood of other groups, and when blood of group A or B is matched with AB recipients The major side should be compatible in these cases.

(*d*) When any other incompatibility is found, the blood groups should be checked If an error in grouping is revealed, new donor blood of the recipient's confirmed blood group is selected for cross match If, however, recheck proves that the original groupings were correct, and that the bloods of donor and recipient are of groups which should be compatible, the most common solution is to determine the exact Rh type of the recipient and to select blood of identical Rh type for repeat crossmatching

SLIDE TEST CROSSMATCH

The action of isohemagglutinins on groups A, B, and AB cells is best demonstrated at lower temperatures, while the effect of Rh anti

CENTRAL SUPPLY ROOM MANUAL, AMERICAN NATIONAL RED CROSS[1]

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[1] Reprinted from *Central Supply Room Manual* National Blood Program Nursing Service American National Red Cross Washington D C ARC 1714 Rev February 1951

clearly demonstrated in high protein media The slide test described below provides adequate protein concentration to disclose blocking antibodies

(*a*) Inactivate donor's and recipient s sera as described in test I(*a*)

(*b*) Dilute donor's and recipient's sera with bovine albumin solution To 1 part of serum add 2 parts of 30 percent bovine albumin

(*c*) Major side Place 1 drop of inactivated recipient's serum fortified with bovine albumin on a slide and add 2 drops of cells from donor s clotted blood sample Mix cells and serum thoroughly and spread over most of the area of the slide

(*d*) Minor side Place 1 drop of inactivated donor's serum fortified with bovine albumin on a second slide and add 2 drops of cells from recipient's clotted blood sample Mix as for major side

(*e*) Place slides in contact with a glass surface which is both lighted and heated to approximately 45°–50° C Observe for agglutination while rocking slowly on the glass surface If there is incompatibility due to Rh or other type of blocking antibody, agglutination will appear in about 2 minutes

Interpretation

If any major side incompatibility is demonstrated, the blood must not be given Minor side incompatibilities may be ignored in extreme emergencies Otherwise, when incompatibility not explainable on an ABO basis is found, sera of the donor and recipient should be studied for Rh Hr or other blocking antibody

should be accessible to the loading platform and to the central supply room A sink for washing equipment to be repacked, and storage space for nonsterile supplies used on the mobile units, are practical additions to this room

II Organization

A working supply of expendable items should be kept in the central supply room It has been found practical to withdraw these items from the general stockroom on the basis of the amounts needed for 1 to 2 weeks. These should be stored in the most convenient spaces along the procedure belt line The shelves and doors of cupboards should be labeled, for this is a helpful means of keeping each item in a specified place and of finding supplies if volunteers are assisting in the central supply room

Because there is a rotation of staff members through this department, it is essential that detailed instructions be maintained of procedures followed in processing supplies, the method of reordering equipment, the source of supplies, and the suggested quantities to have on hand A list of servicing agents for the various items of non expendable equipment should be available and the date these items were last cleaned or serviced should be recorded The administrative procedures established for contacting these agents should be clearly defined

The necessity for meticulous housekeeping of the room cannot be overemphasized Special care must be taken to keep this room free from insects

III Personnel

It is important that specially trained personnel supervise the care and preparation of all equipment that comes in contact with the donor and with the blood itself

In general, one member of the nursing staff should assume responsibility for the operation of the central supply room The basic qualifications for the nurse selected for this duty are comparable with those required for the other nursing staff members The nurse in charge of the central supply room should be alert, capable, and dependable, with a consciousness of the responsibility delegated to her It is also recommended that she have an understanding of methods of teaching and supervision, for the entire nursing staff should be rotated through the central supply room for duty under her supervision, and should be thoroughly familiar with all the details of the procedures performed there

In addition to the above professional and personal qualifications, it is desirable that the nurse have some special technical experience in the establishment and maintenance of central supply rooms

The Central Supply Room

A central supply room in which high standards of technique are rigidly enforced and adequate inventories of sterile nursing supplies are maintained is an integral part of a blood center A successful program is dependent upon the distribution of safe bottles of blood, collected by equipment that is sterile and pyrogen free This can be accomplished by providing modern apparatus, training carefully selected personnel, and maintaining close supervision of the activities

I Physical Facilities

The size of the room needed for the cleaning and preparation of donor equipment will vary according to the number of bloods to be collected daily in the program There are certain factors that should be considered for any room designated for this purpose

A The equipment should be arranged so that the soiled supplies are washed, wrapped, and autoclaved in a belt line process

B Adequate closet and counter space for handling supplies should be provided The closets should have tightly fitting doors, the counters should be at the proper working height, and toe space and knee space supplied in places where the worker will be standing or sitting A few drawers should be planned for, but the majority of the storage space should be in the form of cupboards Adjustable shelves are an advantage and the depth of the shelves should be sufficient to carry such items as needle sharpeners, conductivity meters, and the like

C The distilling apparatus should be placed so that the reservoir is over or adjacent to a sink

D Sufficient electrical outlets should be provided to allow for adequate lighting and make it possible to use electrical equipment where practical in the procedure belt line

E Traffic through the central supply room should be at a minimum Certainly this space should be isolated from the general center traffic so that no one in street clothes should have to use it as a passageway

F Double, deep sinks are the most practical for this room Swivel faucets for hot and cold water and closures for the drains should be provided

G The covering for the floor, counters, and tables should be of durable materials that are easily cleaned and tightly sealed

H A separate room for the packing of mobile unit boxes should be planned where this is possible This feature is especially important if two or more mobile units are anticipated The counters provided in this room should accommodate an opened mobile unit box and should be of the proper working height for packing This room

1 Cutting and preparation of new tubing
2 Preparation of used tubing for resterilization
3 Preparation of needles as assigned
4 Assembling and wrapping donor sets.
5 Preparation of syringes for sterilization
6 Preparation of containers and other equipment for sterile supplies.
7 Such actual sterilization procedures as they are trained to do, under close supervision

Principles of Sterilization

Although there are several physical agents that are capable of destroying bacteria, saturated steam under pressure has been proved to be a most reliable means Therefore, this is the method used for sterilization of supplies for the blood program There are certain factors that influence the effectiveness of saturated steam and these must be considered in the establishment of central supply room procedures.

I FACTORS INFLUENCING THE EFFICIENCY OF STEAM STERILIZATION

A The period necessary to heat the entire contents of the largest package must be known

Size, shape, and internal arrangement of package determine the speed of penetration Saturated steam must contact the entire contents of the package within 14 minutes after the temperature of the fluid in exhaust line reaches 250° F A small, not too tightly wrapped package is most satisfactory for the accomplishment of this principle

B The sterilizer must be loaded to provide a horizontal path for the escape of air

All supplies must be placed in the autoclave to permit air to escape downward All cans and trays must be placed on their sides so that the containers can be filled with steam and the heavy air is not pooled within them

C The quality of the steam used in the sterilizer must be known

Saturated steam is necessary to create a temperature suitable for sterilization When the chamber has been filled with saturated steam, the thermometer at the exhaust line will reach 250° F , if pressure gauge has been set properly This thermometer is an indicator of the quality of steam within the chamber

D The period of continuous exposure to saturated steam must be known

The use of a reliable interval timer is essential Individual watches or clocks should not be depended upon for accurate measurements of time

A Description of activities of nurse in charge of central supply room

1 Establishment and maintenance of high standards of technique in the preparation and sterilization of technical supplies used by the nurses All procedures established must be approved by the medical director

2 Supervision of activities of central supply room

3 Training of nursing and other personnel in established central supply room routines and procedures

4 Preparation and sterilization of equipment and supplies for the safe withdrawal of blood

5 Packing mobile unit boxes.

6 Operation of the sterilizers and still in collaboration with the laboratory technicians.

7 Preparation of solutions as necessary for the cleaning of equipment and the performance of venipuncture.

8 Maintenance of an adequate amount of equipment and supplies as needed for the examination of the donor and performance of venipuncture in both center and mobile unit

9 Continued study in new and improved methods and techniques in central supply room activities with constant evaluation of the procedures performed in the department

10 Establishment of continual checks on items of equipment to insure adequate performance by them

11 Cooperation with laboratory personnel in the sterilization of supplies.

B Use of volunteers and other lay personnel

There is no reason why certain details of these activities cannot be delegated to volunteer nurse s aides or to other lay persons if authorization for employment has been granted and provided definite instructions and on the-job training are combined with careful supervision by the central supply room nurse If volunteer nurse s aides are utilized, it must be remembered that each aide assigned to the central supply room must receive orientation and instruction in the procedures that may be done by her in the department Under no circumstances should a lay person alone be given the responsibility for the central supply room. She should always work under the direct supervision of a nurse. A list of the duties that might be performed by volunteer nurse s aides, or other authorized lay personnel, subject to inspection by the nurse, would include [2]

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[2] See also ARC 1712, unit IV p 7

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3. Preparation of needles as assigned
4. Assembling and wrapping donor sets.
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* See also ARC 1712 unit IV p 7

(2) Muslin wrappers should be of double thickness and should be laundered after each use to preserve fibers

b Steam permeable paper

(1) Certain papers have been manufactured that do have the quality of steam penetration At the present time this paper is too expensive for packaging all goods There may be advantages in its use for selected items

(2) Kraft brown paper is not recommended for wrapping because of the questionable steam penetration that occurs with this type of material

c Sterilizing drums

(1) Steam penetration is severely retarded by the use of drums.

(2) Drying is accomplished with difficulty

(3) Metal dressing drums are expensive, noisy and cumbersome They contribute little to aseptic technique and should not be used [4]

3 Method of wrapping

a Wrappers should be large enough to be securely fastened around article to be sterilized

b Double thickness wrappers should be used and either ties or twine should be used for fastening Pins are often the cause of tears and holes in wrappers

c Articles should be wrapped so that there are four thicknesses of muslin protecting articles from dust, but unnecessary folds of material should be avoided since these merely prolong the period before steam can penetrate goods

4 Use of sterilizing controls in packages

a The value of commercial sterilizing controls is dubious It should be remembered that when they are used the interpretation of the results is a subjective decision, and that often the controls are not 100 percent accurate Emphasis should be placed rather on the procedure of sterilization, being certain that it is foolproof and adheres rigidly to the principles of steam sterilization

B. Loading autoclave

1 The autoclave must be loaded in such a way as to insure circulation of steam around each package Do not overload chamber, especially when sterilizing donor sets The sets should be placed loosely enough in the wrappers and in the autoclave so that the tubing does not become packed into a tight mass

2 Place all flat packages on edge and all containers on their sides with the tops removed Goods should be so placed that the air

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[4] Walter op cit p 72

E Reliable personnel must be used

F There must be adequate sterilizer capacity for the quantity of work to be done

There is no short cut in autoclaving Each load can consist of a certain amount of supplies only, and every load requires a minimum amount of time The quantity of goods to be sterilized daily must be proportionate to the number of loads that can be run daily

G The sterilizer must be in good condition

The sterilizer must be properly cleaned and periodically inspected by someone who understands the maintenance of the specific design of the equipment being used

H *Legible, clearly visible instructions for the operation of the* special sterilizer should be framed and mounted in a conspicuous place

Standardized operation is necessary to insure a constant supply of sterile goods

I A continuous check on the sterilizing process must be made

Use of sterilizing controls, such as Diacks, is of some benefit, as is an occasional, carefully controlled culturing of sterile goods However, both these procedures have their limitations

"Foolproof technic, trained personnel and safe physical minima of time and temperature are more desirable and practical than periodic bacteriologic tests"[3]

II Packaging Supplies for Steam Sterilization

In preparing supplies for autoclaving, it is essential that the articles be free from organic material and oil and grease These substances protect bacteria from microbicidal action Care must also be taken to be certain that all items to be sterilized have access to moisture during the autoclaving process This is accomplished by placing goods in the autoclave in such a way that direct contact of the moist heat is assured, or by leaving a small quantity of distilled water inside articles such as tubing and needles when the small orifices may make penetration by the steam somewhat difficult. (See procedures for the cleaning of specific supplies)

A Protective covering for goods

1 Function of covers

a Protect materials from contact contamination

b Act as a dust filter

c Allow for ready passage of steam

2 Types of wrappers

a Muslin

(1) Good quality muslin is the most suitable material

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[3] Walter Carl W *The Aseptic Treatment of Wounds* p 91

Pyrogens are soluble carbohydrates produced by the growth of non pathogenic bacteria in water or on dust. Pyrogens, when injected into the blood stream, are capable of causing untoward reactions characterized by chills and fevers. It is essential that all solutions and supplies used in the collection of blood be free from these pyrogenic substances as well as from bacteria and foreign substances

Since pyrogens are thermostable, articles that are to be sterilized must have no pyrogens present when they are placed in the autoclave. The bacteria responsible for producing pyrogens can be killed by saturated steam under pressure, but pyrogens are not destroyed by exposure to steam Pyrogens can be removed from such equipment if sufficient pyrogen free distilled water is used for rinsing However, it is necessary that these rinsed supplies be promptly autoclaved since bacteria may grow in the small particles of moisture clinging to the articles, thereby producing pyrogens No more than 4 hours should elapse between final rinsing and sterilization If goods are not to be sterilized, they must be dried thoroughly before storing

Pyrogen free distilled water may be obtained by the use of a well designed still. The purity of the distillate is naturally dependent upon the careful operation and maintenance of the apparatus Since bacteria can grow in distilled water, it is important that the distillate be kept in a dustproof container and that water distilled for a longer period than 4 hours be considered unsafe for use in rinsing equipment and in preparing solutions Only a working supply of distilled water should be collected and the carboy should be drained as soon as there is no immediate use for the distillate.

Testing for pyrogens

The only method suitable for identifying the actual pyrogen content is a biological test using rabbits This is a test that necessitates careful control and handling of the test animals

The efficiency of a still can easily be checked by the use of a conductivity meter, which determines the electrical resistance of the distillate Electrolytes in distilled water indicate a contamination with tap water and it may then be assumed that pyrogenic substances are present also It must be remembered that when the conductivity meter is used, it indicates the purity of the distilled water at that particular time only In order to be assured that the distillate is constantly free from electrolytes, a continuous record of the conductivity of the water would be necessary (For use of conductivity meter, see procedure, p 170)

will drain from the containers and not remain pooled within them This will facilitate steam penetration

3 Avoid placing goods so that they will be against the door of the chamber when the door is closed Condensation of steam may occur and cause these packages to be wet even when the drying cycle has been completed

4 Solutions should always be autoclaved separately from other supplies

C Operation of steam sterilizer (See procedure, p 170)

D Storage of sterile goods

1 Each package should be labeled with the date of sterilization as it is removed from the autoclave

2 Sterile and unsterile packaged goods must never be stored on the same storage shelf The counter where each is placed must be clearly identified by conspicuous signs

3 Supplies that are removed from the autoclave while they are still warm should be placed on padded surfaces so that condensation will not cause dampening of the wrappers

4 Packages wrapped in four thicknesses of muslin, and properly sterilized, may be considered sterile indefinitely providing the following precautions are taken

a Careful rotation of goods is established so that the goods bearing the oldest date are used first

b Sterile packages are stored in a clean, closed closet and allowed to remain undisturbed

c Articles packed for mobile units, or otherwise handled frequently, and not used within 1 month after sterilization should be re autoclaved

5 A central storage closet for sterile goods will probably afford the soundest system of rotation If supplies are kept in the utility room or in the mobile packing room, the inventory should be kept low and a careful check of the date of sterilization should be made

E Period of sterilization

1 Goods should be sterilized for 30 minutes at 250° F, 15 pounds pressure

a Timing should begin only when indicating thermometer reaches 250 F

b Timing should be done with an interval timer

c Thirty minutes sterlizing time allows 10 to 15 minutes for steam penetration of the load, and 13 to 15 minutes for destruction of bacteria and spores

CARE AND STERILIZATION OF NEEDLES

I General instructions

A Be certain that the entire needle, as well as the lumen of the cannula, is washed with detergent solution and rinsed with distilled water

B When flushing needles with distilled water, flush fresh water through each needle

II Care and sterilization of hypodermic needles

A Needles should be placed in a container after use and submerged in water until they are to be cleaned

B Wash and force flush with hot solution of detergent

C Rinse and force flush with pyrogen free distilled water

D Test needle for sharpness and for temper

E Place needles in constriction tubes, plug ends with cotton, place constriction tubes in wire basket

F With tubes in horizontal position, place basket in autoclave, and sterilize at 250° F, 15 pounds pressure, for 30 minutes

G Needles that are cleaned and not sterilized immediately should be placed in the hot air oven to dry before storing Just prior to sterilization, steps C, D, E, and F should be repeated

III Care and sterilization of hose hub donor needles

A Needles should be rinsed with cold water immediately after use and kept submerged in water until they are to be cleaned

B Cleanse hub of needle carefully with dampened applicator

C Hold needle under running water and stylet vigorously with close fitting stylet

D Wash and force flush with hot solution of detergent

E Rinse and force flush with pyrogen free distilled water

F Leave lumen moistened with distilled water, place needles on clean towel, and assemble promptly with tubing for donor set

G If needles are not to be used immediately, they should be dried in the hot air oven and placed according to size in clean, covered containers Just prior to sterilization, steps E and F should be repeated

IV Care of new needles

A Wash and force flush with hot solution of detergent

B Rinse and force flush with pyrogen free distilled water

C Follow procedures as outlined above

V Sharpening needles

A. *Donor needles* may be sharpened on the electric machine, hypodermic needles can be made usable on an Arkansas oilstone Care and experience are necessary to repair a dulled or burred point The original bevel should be maintained and a sharp cutting point produced

Procedures for Cleaning and Sterilizing Supplies

GENERAL CONSIDERATIONS IN WASHING ARTICLES

I All articles prepared for sterilization must be thoroughly clean—free from all organic material and oil and grease

II The detergent selected for cleaning supplies should be one that leaves no insoluble deposits on the surface of the articles being washed The commercial synthetic detergents have proved very effectual for cleaning The amount of detergent used should be consistent with the recommendations made on the label by the manufacturer Sudsing detergents should not be used in mechanical glass and needle washers

III Glassware should not be dried with a towel but allowed to drain or to be dried in the hot air oven

CLEANING AND STERILIZING GLASSWARE

I Syringes

A Rinse syringe promptly with cold water after use and dis assemble

B If blood has clotted within the syringe, a blood dissolving substance may be used as a soak

C Wash well in hot solution of detergent Inspect for visible cleanliness

D Rinse well with pyrogen free distilled water

E Match numbers on barrel and plunger Wrap parts carefully in gauze, and then in muslin wrapper

F Autoclave at 250° F , 15 pounds pressure, for 30 minutes

G Date and store in designated place

Note—If large numbers of syringes are used a rubber band should be fastened loosely about the separated parts of a syringe immediately after use thereby keeping parts together during cleaning process The band should be removed when the parts are wrapped

The use of a wire strainer or dipping basket in washing many syringes is advantageous The syringes can be placed in the strainer and the strainer then plunged several times up and down in the hot detergent solution This prevents breakage and handling during this step of the cleaning procedure

II Care of needle vials

A Remove rubber stoppers or cotton plugs

B Wash in hot solution of detergent

C Rinse well with tap water

D Rinse well in pyrogen free distilled water

E Place in wire basket and dry in hot air oven.

III Cleaning general glassware

A Wash well in hot solution of detergent

B Rinse well in hot tap water

C Rinse in distilled water

D Allow to drain or dry in hot air oven

4 Place in covered pail of water to be returned to central supply room

B In central supply room

1 Attach tubing to jet rinsers and allow cold water to run through tubing for 20 minutes

2 Wring tubing again through hand wringer

3 Force flush with cold water to remove any loosened clots

4 Flush with at least ½ pint of pyrogen free distilled water

5 Flush with pyrogen free normal saline, coating inner lumen with solution Allow some saline to remain in tubing

6 Cut ends of tubing as necessary to insure close fit with needles

7 Place on clean towels and promptly assemble with donor needles Tubing must be autoclaved within 4 hours after completing step 5

Note —If it is not possible to thoroughly flush tubing immediately after use tubing should be soaked in cold water and when returned to central supply room it should be force-flushed clots loosened flushed and the procedure described above for the preparation of new rubber tubing should be followed.

CARE OF DONOR SETS (RUBBER TUBING)

Equipment

1 17G 1½ inch needle
1 15G 1 inch needle
1 piece of tubing 16 inches long
2 double constriction tubes—each having one end plugged with cotton, other end with fitting rubber cap
Gauze squares
Wrapper

A Prepares needles, tubing, and glassware as suggested above

B Assemble donor set by placing a 17G needle on one end of tubing and a 15G needle on the other Be certain that the needles are in good condition

C Place needle vials over each needle

D Wrap 10 donor sets in a package Wrap gauze in such a manner that rubber surfaces are not in contact and that rubber tubing is not folded Place sterilizer controls in center of package Muslin wrapper should be applied securely to prevent entrance of insects or dust during storage period

E The sets should be autoclaved as soon as possible after assembling, and the time elapsing between final rinsing and sterilizing must not exceed 4 hours.

F Autoclave at 250° F, 15 pounds pressure, for 30 minutes

G Date and store in closed closet

B A light oil such as household sewing machine oil should be used to lubricate the oilstone

C After being sharpened, the needles should be cared for as outlined in the above procedure for new needles

CARE OF TUBING

I Plastic tubing

A Cut tubing into lengths These lengths should be as short as possible since this tubing is not re u ed, yet they must be long enough to be practical for the type bottle holder used in the center

B Rinse lengths of tubing with pyrogen free distilled water

C Place on clean towel and promptly assemble with needles

Note—When reel of tubing is not being used it should be stored in dust proof closet

II Rubber tubing, new

A Wash well in solution of detergent

B Cut tubing into lengths

C Tie 15 pieces of tubing together at one end

D Place 5,000 cc of 5 percent solution of sodium carbonate in enamel or stainless steel bucket

E Immerse tubing, putting tied ends in solution first and having solution fill lumen

F Weigh tubing down so that it stays completely submerged during boiling

G Place bucket on electric hotplate and boil tubing for 20 minutes

H Rinse outside of tubing well with tap water Attach tubing to water jet and allow cold water to run through tubing for 20 minutes

I Flush each piece of tubing with at least ½ pint of distilled water

J Flush each length with pyrogen free normal saline, coating inner lumen with solution Allow some moisture to remain in tubing

K Place on clean towels and promptly assemble with donor needles Tubing must be autoclaved within 4 hours after completing step J

III Rubber tubing, used

A Immediately after use

1 Disassemble tubing from other equipment and force flush with cold water Available tap water must be essentially free from pyrogens

2 Use small hand wringer attached to bucket—wring tubing through this to loosen clots.

3 Force flush again with cold water

sterilization 10 to 25 blades, depending upon anticipated operations, may be threaded through one strip

F Roll gauze and place in small screw top jar or other suitable container for sterilization

G Wrap jar (with lid removed) and lid in muslin

H Autoclave for 30 minutes at 250° F, 15 pounds pressure Allow to dry thoroughly after sterilization

I Date and store in designated place

STERILIZATION OF 2 x 2 SPONGES

I Packaged

A Open one end of paper wrapper for steam penetration

B Place one to two packages in muslin wrappers

C Autoclave at 250° F, 15 pounds pressure, for 30 minutes

D Date and store in space designated for sterile goods

II In dressing jars

A Wash jar with hot solution of detergent Rinse with tap water and dry

B Separate sponges and fill cans approximately ⅔ full

C If cans are to be used in center operations within the next day, they may be autoclaved unwrapped Gauze may be tied over top of cans with cover loosely in place, providing care is taken to see that cover falls against gauze and away from top of can when placed in autoclave

D Cans of 2 x 2 s to be used on mobile units or stored should have the top removed and both can and top wrapped in muslin

E Place can on its side in the autoclave Sterilize at 250° F, 15 pounds pressure, for 30 minutes

F Date and store in closed closet designated for sterile goods

STERILIZATION OF APPLICATORS

I Packaged

A Place 50 applicators in muslin wrapper

B Autoclave at 250° F, 15 pounds pressure, for 30 minutes

C Date and store in space designated for sterile goods

II In applicator jars

A Wash applicator jar with hot solution of detergent Rinse with tap water and dry

B Fill jar with applicators, cotton wrapped ends placed downward

C Prepare jars for autoclaving as described above

D Sterilize at 250° F, 15 pounds pressure, for 30 minutes Date and store

Care of Donor Sets (Plastic Tubing)

Equipment

1 piece plastic tubing
1 17G 1½ inch length
1 15G 1 inch length
2 needle protectors (pieces of plastic tubing)
3 inches—diagonally cut on one end, other end sealed
4 inches—diagonally cut on one end, cotton plugged on other
Wrapping material

A Prepare needles and plastic tubing as outlined above

B Attach a 17G needle to one end of tubing, a 15G needle to the other Be certain that needles are in good condition

C Donor sets must be assembled while the tubing and needles are still moist with distilled water Dip 15G needle into fresh pyrogen free distilled water so that fluid level in the tubing shows an inch beyond the hub Leave this water in the tubing

D Place 3 inch protector over 15G Place 4 inch protector over 17G needle

E Wrap sets as described under 'Care of Donor Sets (Rubber Tubing)' or place 10 sets in fold of Dennison's Government Board moistened with distilled water Wrap cardboard in Dennison's steam permeable paper Seal with Vinylite adhesive

F Autoclave within 4 hours of final rinsing Autoclave at 250° F, 15 pounds pressure, for 30 minutes

G Date and store for 3 days before using This will allow tubing to resume its normal resiliency

Care and Sterilization of Hemoglobin Lancets

Bard Parker blades

A Immediately after use, wipe blade with dry sponge to remove blood Place carefully in a dry container so that cutting edge is not dulled

B If blades have become discolored, they may be
1 Scrubbed with an abrasive
2 Washed well with a hot solution of detergent
3 Rinsed well with distilled water

C Resharpen blades as necessary Rinse well after resharpening

D Dry blades carefully

E Fold piece of gauze into long strip approximately 1½" wide Thread blades into gauze, being certain that tops of blades extend above strip in order that blades may be easily removed after

Size	Center	Mobile Unit	Total
9 x 9 inches with pockets	100	100	200
9 x 9 inches	100	100	200
20 x 20 inches	200	200	100
28 x 28 inches	200	300	500
40 x 18 inches	2 0	3 0	600
Knee sheets	50	50	100
Donor gowns	25	25	50
Card table covers	35	35	70

PREPARATION OF SOLUTIONS

I Sterile normal saline

Formula

Sodium chloride, 8 5 gm

Distilled water q s. ad , 1,000 cc.

Procedure

1 Weigh the required amount of sodium chloride (8 5 *gm per* 1,000 *cc of distilled water*) in an aseptic manner

2 Clean Fenwal flasks[*] as suggested (See Cleaning and Sterilizing Glassware) Just before using, flush flask bushing and stopper with pyrogen free distilled water

3 Place weighed sodium chloride in flask and fill with required amount of freshly distilled water

4 Fit a clean rubber bushing into mouth of container and *turn down skirt Insert channeled stem of the stainless steel stopper* into the aperture of the bushing so that grooved portion permits the escape of air and steam from container

5 Label

6 Autoclave at 250° F , 15 pounds pressure, for 30 minutes

7 After sterilization is completed, turn operating valve to "off" and reduce chamber pressure to zero Open chamber door slowly

8 Remove container carefully, without agitation, and immediately push metal stoppers into bushings to complete seal

9 Date Contents of Fenwal flask may be considered sterile as long as a water hammer click is present, indicating the flask has been hermetically sealed

II 5 percent sodium carbonate solution

Formula

Sodium carbonate, 50 gm

Distilled water q s ad , 1,000 cc

III 70 percent alcohol

Formula

Ethyl alcohol (95 percent at 25° C), 815 cc

Distilled water (cold) q s ad , 1,000 cc

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[*] Red Cross Blood Centers use *only Fenwal flasks for solution preparation* and storage

Other CSR Operational and Organizational Activities

DETAILS REGARDING WRAPPER

I General instructions

A Muslin wrappers should be made of double thickness Protection of sterile articles by four thicknesses is obtained by the method of folding the wrapper

B Allow 1 to 2 inches for shrinkage when cutting wrappers Sizes given below are those required after laundering

II Sizes needed

9″ x 9″—stitched with pockets for two 2 cc syringes



9″ x 9″—without pockets
For Bard Parker blades in jars
20 cc syringes
Alcohol and iodine jars

20″ x 20″
For Forcep jars
Extra sponges
Extra applicators

28 x 28
For Dressing jars
Applicator jars
Suture sets

40 x 18
For Donor sets

III Other muslin supplies that may be made
Knee sheets Single thickness squares of 30″ x 30″
Donor gowns Short jackets for donors
Card table covers Single thickness squares of 40″ x 40″

IV Number of wrappers needed

It is difficult to furnish formulae for supplies since the pattern of operation varies somewhat with each center The number of wrappers needed will depend on the number of operations held weekly, the number of mobile units attached to each program, and the efficiency of the laundry service provided to the center The following figures are applicable to a program with a daily center operation of about 100 bleedings plus one mobile unit with daily operations of 100 bleedings and a semiweekly delivery of laundry

Note—If rubber tubing is attached to drain bib for draining and tubing extends into sink, this piece of tubing should be removed when evaporator has been drained.

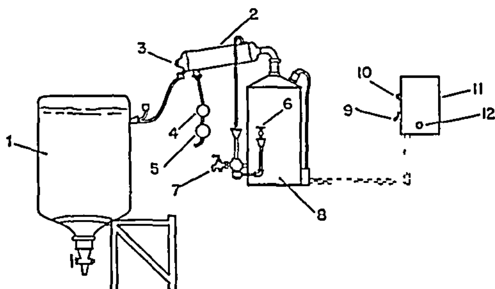
IV Points to be emphasized in operation of still

A Line from condenser to carboy should be entirely of tin tubing, free from kinks or traps. Tubing should be inserted into carboy through a tight fitting rubber stopper.

B The still should be run at only about three quarters of its capacity to prevent bacteria or pyrogens from being carried from raw water in evaporator to the condenser.

C The still should be disassembled and cleaned at regular intervals as recommended by the manufacturers.

D When the still is started in the morning, the first 200 to 300 cc. of distillate should be discarded as it may contain dust particles.



1 Pyrex Carboy
2 Condenser
3 Vent
4 Throttle Valve
5 Shut-off Valve
6 Deconcentrator Valve
7 Bib Drain
8 Evaporator
9 Control Space
10 Manual Reset Button
11 Low Water Control
12 Pilot Light

WATER DISTILLING APPARATUS

BARNSTEAD STILL

IV 2 percent aqueous iodine
Formula
Iodine, 20 gm
Sodium iodide, 24 gm
Distilled water q s ad, 1,000 cc.

V 50 percent solution of soap
Formula
Tr green soap, 500 cc
Distilled water q s ad, 1,000 cc

OPERATING INSTRUCTIONS FOR BARNSTEAD WATER STILL

I Care of pyrex carboy before attachment to condenser

A Rinse carboy (1) well with potassium dichromate solution *or* fill with solution of Calgolac or Glim and allow to stand overnight

B Rinse thoroughly with tap water

C After carboy has been attached and still inspected for installation, allow still to run for a day The carboy should be allowed to fill with the freshly distilled water and then emptied and refilled continuously during the day that the still is being flushed with the water

II Initial operation of the still

A Close bib drain (7)

B Open throttle valve (4) on cold water supply one fourth turn, open shut off valve (5) on cold water supply wide

C As soon as water in evaporator (8) reaches maximum level, push control switch (9) to "on," and press manual reset button in Distillate will soon issue from condenser (2)

D When still has been running for about 15 minutes, regulate throttle valve so that a slight wisp of steam issues from condenser vent (3) This valve should be set permanently and still cold water supply may be turned "on" and "off" by the shut off valve

E Deconcentrator valve (6) on evaporator should be opened at approximately one and one half times the rated capacity of the still, when the still is in operation If water is temporarily hard, see instructions from the Barnstead Still Company

III To discontinue operation

A Turn control switch to "off"

B Turn water supply valve to "off"

C Open drain bib

D When no more distilled water is needed from the carboy for the day's operation, reservoir should be drained No water should remain in reservoir overnight.

B With operating valve turned to "off," turn switch handle to "on"

C Sterilization process cannot begin until jacket pressure is 15 to 17 pounds

II Load chamber

III To sterilize material

A Securely fasten door of chamber. With jacket pressure at 15 to 17 pounds, lock chamber door by turning operating valve to "ster"

B Do not attempt to time load until indicating thermometer shows 250° F. When thermometer reaches 250° F, time load for 30 minutes. Interval timer should be used.

IV To exhaust chamber (for all supplies but solutions)

A At end of exposure period, turn operating valve to "exh" until chamber gauge reaches zero pressure.

B Turn operating valve to "dry" for a period of 5 to 15 minutes *depending upon size of load*

C Turn operating valve to "off" and slowly open the door of the chamber. Leave door slightly ajar for a few minutes before removing goods.

V To exhaust chamber when sterilizing solutions

A At end of exposure period, turn operating valve to "off" and wait until chamber gauge shows zero pressure

B Open chamber door slowly to prevent violent ebullition Fenwal flasks must have the steel plungers pushed into the bushings immediately before the solution cools

VI If sterilizer is not to be used again, turn switch to "off"

Note—Reservoir should be replenished after each run. Always fill with water to slightly below "full" mark.

OPERATION OF AUTOCLAVE EQUIPPED WITH CYCLOMATIC CONTROL

I To heat jacket

A Open valve to reservoir until indicator shows water level to be slightly below "full" mark

B Turn switch handle to "on"

C Allow pressure gauge (lower right hand corner of panel) to register 15 to 17 pounds pressure

II Load chamber and securely fasten door of chamber

III Set selector (upper left hand corner of panel) to "dry" for wrapped goods, "slow exhaust" for solutions

IV Set timer (upper right hand corner of panel) to 30 minutes

V Turn control (center of panel) to "sterilize"

VI All phases of the sterilizing cycle will be automatically completed by control. Visual indication of progress is provided by three lights

Instructions for the Use of the Conductivity Meter

Procedure for testing electrical resistance of distilled water

A Carefully rinse a pyrex container or beaker, a water bath thermometer, and the cell of the meter with freshly distilled water

B Collect sample of water in rinsed container

C Connect cell (7) to terminals (4) of water checker

D Plug line cord (5) into electrical outlet of proper voltage

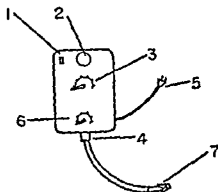
E Determine temperature of the solution

F Insert cell into water, moving it up and down several times under the water level to remove air from cell casing

G Set lower dial (6) of meter to the proper temperature of the water

H Turn switch (1) "on"

I Rotate upper dial (3), observing the action of the indicating eye (2) When the darker segment of the eye reaches its widest angle the wheatstone bridge is in balance The dial will then directly indicate the number of sodium ions present in terms of parts per million



1 Switch
2 Indicating Eye
3 Upper Dial
4 Terminals
5 Line Cord
6 Lower Dials
7 Cell

CONDUCTIVITY METER

Note —Water containing more than two parts per million of sodium chloride ion should be rejected for use

The conductivity meter should be used daily It is recommended that a record be kept of these daily readings

Between periods of use the cell should be stored in a glass container of distilled water and kept in a dust free closet.

Operation of the Electric Steam Sterilizer

I To heat jacket

A Open valve to reservoir until indicator shows water level to be slightly below "full" mark.

THE PLACE OF SALINE IN TREATMENT OF INJURIES WITH SHOCK[1]

The following recommendations regarding the proper role of *saline* in the treatment of traumatic and burn shock have been prepared by the subcommittee on Shock of the National Research Council for the guidance of all civil defense health and special weapons defense services.

Saline for Parenteral Administration

The proper place of intravenous saline in the treatment of injured persons in shock can be adequately defined on the basis of existing knowledge

A large amount of work has been done on this problem, over many years both experimentally, clinically, and in battle casualties during World War II While certain questions remain unanswered and some differences of opinion persist, it is believed that a general statement regarding the procurement and stockpiling of these solutions can be made to which most authorities if not all, will agree, and which will permit appropriate action to be taken by the Armed Forces and civil defense agencies

The function of saline as an element in the bodily economy is essentially kinetic and not static Saline tends to move through the body rather than remain in it Normally a salt solution is rapidly distributed through the body fluids following intravenous administration and then excreted through the kidneys

In the presence of dehydration, blood loss, and other conditions where there is a net loss of tissue fluid, additional salt and water is needed for replacement over and above that required for urinary excretion The same applies to conditions in which extra fluid accumulates in tissues, as in the edema of burns, crush injury, infection and the like Sweating also uses up electrolytes and water When these volumes are satisfied, again the basic need for added salt and water is for purposes of urinary excretion

[1] Prepared by the Subcommittee on Shock Division of Medical Sciences National Research Council Washington D C

VII At completion of cycle, buzzer will sound Control should be turned to "off"

VIII Open door and leave slightly ajar for approximately 5 minutes before removing load

BIBLIOGRAPHY

AMERICAN NATIONAL RED CROSS *Technical Manual, National Blood Program* ARC 1702

DEGOWIN, ELMER L, HARDIN, ROBERT C, and ALSEVER, JOHN B *Blood Transfusion* Philadelphia W B Saunders Company, 1949

UNDERWOOD, WEEDEN B *Textbook of Sterilization* Chicago Lakeside Press, 1941

WALTER CARL W *The Aseptic Treatment of Wounds* New York The MacMillan Company, 1948

WHITE C S and WEINSTEIN, J *Blood Derivatives and Substitutes* Baltimore Williams & Wilkins, 1947

abdominal injuries or (4) when there is, for any reason, a tendency to pulmonary edema

With the above reservations, measures which will provide oral saline for injured persons who are either not in shock or are in mild shock only, are recommended

Indications and Dosage Schedule

The following brief schedule of suggested therapy gives an approximate indication of materials that can be used, and the amounts required

Parenteral (intravenous) saline isotonic—NaCl, NaCl and glucose, NaCl and $NaHCO_3$ Na lactate, Ringer, Ringer lactate, etc.

(*a*) Indications

(1) Dehydration saline solutions clearly indicated, may provide complete relief of condition Amounts one to three liters, sometimes much more in severe dehydration, as in diabetic acidosis, cholera, etc.

(2) Shock due to or associated with massive blood loss useful as supplement to plasma or blood, especially if there is added dehydration, or to sustain circulation for one half to one hour while blood or plasma is being obtained One to two liters of saline can maintain some increase in blood volume and cardiac output for one to two hours. Too much intravenous saline may overhydrate tissues, will eventually lead to venous congestion and pulmonary edema Plasma protein levels will give indications of overhydration

(3) Shock due to burns Saline in larger amounts up to three or four liters daily, may be useful as a supplement to blood or plasma in extensive second and third degree burns, during the first 36 hours after injury Caution after 36 hours, as there is risk of circulatory congestion and pulmonary edema

(4) Continuation therapy When water and electrolytes can not be taken by mouth in quantities sufficient to maintain urinary volume at normal or adequate levels, additional parenterally administered saline may be indicated

(*b*) Contraindications

(1) Congestive heart failure (venous congestion, pulmonary and peripheral edema)

(2) Chest injuries (pulmonary edema)

(3) Burns involving tracheobronchial tree or lungs (pulmonary edema)

(4) Renal shutdown, except where actually due to dehydration or existing shock

In acute shock, when blood or plasma is not available intravenous saline, rapidly administered, can frequently restore and sustain the circulation for brief periods After one or two liters of intravenous saline, a significant increase in plasma volume will be maintained for perhaps an hour or two, as compared with 12 to 24 hours when blood or plasma has been given Saline is also helpful in maintaining patency of vein, needle, and tubing, between transfusions Additional saline has a special place in burns

In the general scheme of therapy, saline is a complement to blood or plasma, and not a substitute for them Parenterally administered saline is needed (1) to provide salt and water for dehydrated tissue depots, (2) to provide salt and water for essential urine volume in all instances where the injured person cannot take sufficient food electrolytes, and water by mouth for these purposes The basic criterion for saline requirement is urine volume, not plasma volume In one clinical situation blood may be needed, and saline not needed at all, in an other clinical situation, saline may be required for days or even weeks after the need for blood has passed In still other situations, glucose *and water may be indicated, rather than saline*

Accordingly, it is apparent that the use of parenteral saline is an essential part of the treatment of the injured person in shock, separate from but complemental to, the use of blood, plasma, or plasma expander

This Committee therefore recommends to the Armed Forces and FCDA the procurement of saline solutions suitable for parenteral administration, for the treatment of wounds and burns, over and above, and separate from the necessary supplies of blood, plasma, and plasma expanders

Saline for Oral Administration

As indicated in the preceding discussion, saline is needed chiefly to supply tissue depots and to provide for urine volume However, saline by mouth, if it can be retained and absorbed, should be as effective as saline by vein Oral saline will be of value therefore as supplementary and continuation therapy in patients who are in condition to tolerate it. It will be of special usefulness in burns of moderate severity or extent, where the patient is not in severe shock, but where the requirement for additional salt and water is large

Under ordinary circumstances oral saline, like all other measures, should be administered only upon the advice of a physician. Used indiscriminately, especially in cases of severe shock, oral saline can do great harm either (1) through vomiting, with additional loss of salt and fluid, (2) through vomiting and aspiration, with the risk of aspiration pneumonia or of immediate asphyxial death, (3) in

abdominal injuries or (4) when there is, for any reason, a tendency to pulmonary edema

With the above reservations, measures which will provide oral saline for injured persons who are either not in shock or are in mild shock only, are recommended

Indications and Dosage Schedule

The following brief schedule of suggested therapy gives an approximate indication of materials that can be used, and the amounts required

Parenteral (intravenous) saline, isotonic—NaCl, NaCl and glucose, NaCl and $NaHCO_3$ Na lactate, Ringer, Ringer lactate, etc

(*a*) Indications

(1) Dehydration saline solutions clearly indicated, may provide complete relief of condition Amounts one to three liters, sometimes much more in severe dehydration, as in diabetic acidosis, cholera, etc

(2) Shock due to or associated with massive blood loss useful as supplement to plasma or blood, especially if there is added dehydration, or to sustain circulation for one half to one hour while blood or plasma is being obtained One to two liters of saline can maintain some increase in blood volume and cardiac output for one to two hours. Too much intravenous saline may overhydrate tissues, will eventually lead to venous congestion and pulmonary edema Plasma protein levels will give indications of overhydration

(3) Shock due to burns Saline in larger amounts up to three or four liters daily, may be useful as a supplement to blood or plasma in extensive second and third degree burns, during the first 36 hours after injury Caution after 36 hours, as there is risk of circulatory congestion and pulmonary edema

(4) Continuation therapy When water and electrolytes cannot be taken by mouth in quantities sufficient to maintain urinary volume at normal or adequate levels, additional parenterally administered saline may be indicated

(*b*) Contraindications

(1) Congestive heart failure (venous congestion, pulmonary and peripheral edema)

(2) Chest injuries (pulmonary edema)

(3) Burns involving tracheobronchial tree or lungs (pulmonary edema)

(4) Renal shutdown, except where actually due to dehydration or existing shock

Oral saline—(Chilled slightly hypotonic salt and sodium bicarbonate solution, or one sixth molar sodium lactate, is more palatable than isotonic sodium chloride)

(*a*) Indications

(1) Dehydration, when and if tolerated by GI tract

(2) Injuries with mild shock One to three liters daily, to relieve thirst and provide fluid and salt

(3) Mild to moderate burns If parenteral fluids are not available, saline, preferably chilled, can be taken by mouth in amounts up to two or three liters daily, providing patient's general condition is fairly good, and there is no risk of aspirating vomitus Patients sometimes vomit oral saline at first, but subsequently are able to retain it.

(*b*) Contraindications

(1) Severe shock or poor clinical condition with risk of vomiting

(2) Abdominal injuries.

(3) Inability to retain oral fluids

(4) Renal shutdown, not due to dehydration or existing shock

In regard to the use of oral saline as a first aid measure by laymen, as suggested in *Health Services and Special Weapons Defense*[2] and in *Emergency Action to Save Lives*,[3] it is again emphasized that adequate first aid instruction must be obtained by all people who intend to undertake first aid measures in civil defense emergencies State and local civil defense health services should urge widespread first aid training and see to it that proper instructions are included regarding the circumstances in which oral saline can be used safely by first aid workers

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[2] *Health Services and Special Weapons Defense* FCDA AG-11-1 par "66-" ,0 p 103

[3] *Emergency Action To Save Lives* FCDA PA 5

Official Civil Defense Publications

The following Federal Civil Defense Administration publications are on sale by the Superintendent of Documents, Washington 25, D C

1 *United States Civil Defense*, 1950, 25 cents, 168 pp The national plan for organizing the civil defense of the United States.

Administrative Guides

1 *Civil Defense in Industry and Institutions*, Pub AG-16-1, 1951, 25 cents, 64 pp Plans for organizing and administering civil defense self protection programs for the Nation s industrial plants, office and apartment buildings, and other institutions.

2 *The Clergy in Civil Defense*, Pub AG-25-1, 1951, 10 cents, 12 pp Guide for the clergy of all faiths for determining their place and function in civil defense

3 *Emergency Welfare Services*, Pub AG-12-1, 1952, 25 cents, 62 pp Guide for developing a program to meet the multiple welfare problems that would arise from enemy attack.

4 *Engineering Services*, Pub AG-13-1, 1952, 15 cents, 25 pp Assists State and local civil defense directors in planning and establishing their engineering services

5 *Fire Services*, Pub AG-9-1, 1951, 15 cents, 27 pp Basic guide to assist States and communities in planning, organizing, staffing, and operating an expanded fire fighting service during periods of war emergency

6 *Health Services and Special Weapons Defense*, Pub AG-11-1, 1950, 60 cents, 264 pp Methods for organization of all basic health and special weapons defense (atomic, biological, and chemical warfare) for State and local civil defense programs

7 *Police Services*, Pub AG-10-1, 1951, 25 cents, 48 pp Basic guide for State and local civil defense officials in organizing and directing police civil defense services

8 *Principles of Civil Defense Operations—Web Defense—Mutual Aid—Mobile Support*, Pub AG-8-1, 1951, 20 cents, 48 pp Basic guide in planning and organizing for mutual aid and mobile support operations

9 *The Rescue Service* Pub AG-14-1, 1951, 15 cents, 32 pp Basic guide for State and local civil defense officials in organizing rescue services and training rescue teams

10 *The Supply Service*, Pub AG-6-1, 1952, 25 cents, 50 pp Assists State and local civil defense directors and supply officials in establishing adequate supply programs

11 *The Warden Service*, Pub AG-7-1, 1951, 20 cents, 48 pp Basic guide for civil defense directors and supervisory wardens in selecting, organizing, training, and equipping the warden service

Public Booklets

1 *Emergency Action To Save Lives*, Pub PA-5, 1951, 5 cents, 32 pp Practical instructions for the untrained person on the emergency care of injured people

2 *Fire Fighting for Householders*, Pub PA-4, 1951, 5 cents, 32 pp Basic information for the householder on how fires start, how they can be prevented, and how to fight fires

3 *What You Should Know About Biological Warfare*, Pub PA-2, 1951, 10 cents, 32 pp Techniques of personal survival under biological warfare attacks

4 *Survival Under Atomic Attack*, 1950, 10 cents, 32 pp Techniques of personal survival under atomic attacks

Technical Manuals

1 *Civil Defense in Schools*, Pub TM-16-1 15 cents, 32 pp A guide and reference for local and State superintendents of schools in organizing and operating programs for the self protection of schools, their physical facilities, staff and students

2 *Fire Effects of Bombing Attacks*, TM 9-2, 1952, 20 cents, 48 pp Summarizes data on World War II bombing attacks and suggests a method of appraising fire susceptibility of cities to minimize the effects of mass fires

3 *Organization and Operation of Civil Defense Casualty Services*, Part I—*The First Aid System*, TM-11-1, 1952, 25 cents, 52 pp Recommends general principles designed to assist key civil defense professional medical personnel in planning and operating a first aid system

4 *Organization and Operation of Civil Defense Casualty Services*, Part III—*Medical Records for Casualties*, Pub TM-11-3, 1952, 20 cents, 31 pp Recommends medical records and forms for uniform use by all States in the handling of casualties resulting from enemy attack

5 *Outdoor Warning Device Systems*, Pub TM-4-1, 1951, 15 cents, 36 pp Data for planning, procuring, and installing public warning device systems for civil defense

6 *Radiological Decontamination in Civil Defense*, Pub TM-11-6, 1952, 20 cents, 31 pp Provides information for all radiological defense personnel and serves as an operations manual for decontamination crews

7 *Shelter from Atomic Attack in Existing Buildings*, Part II—*Improvement of Shelter Areas*, Pub TM-5-2, 1952, 15 cents, 22 pp Offers suggestions to architects and engineers for improving shelter areas.

8 *The Nurse in Civil Defense*, Pub TM-11-7, 1952, 25 cents, 52 pp Assists key civil defense nurses in planning and operating State and local nursing services

9 *Utilization and Control of Streets and Highways in Civil Defense Emergencies*, Pub TM-13-1, 1953, 15 cents, 24 pp Describes the problems involved in keeping selected urban streets and rural highways free from serious congestion in civil defense emergencies and suggests methods of solving these problems

10 *Water Supplies for Wartime Fire Fighting*, Pub TM-9-1, 1951, 10 cents, 16 pp Program for increasing available water supplies to meet the needs of emergency water supply operations during wartime

11 *Windowless Structures—A Study in Blast Resistant Design*, Pub TM-5-4, 1952, $1 00, 165 pp Describes methods and procedures for designing windowless structures or windowless portions of conventional structures, based on the dynamic properties of loading, presents principles, methods and formulas for determining the magnitude, duration, and distribution of atomic blast loads on windowless structures

Technical Bulletins

1 *Construction and Adaptation of Structures for Rescue Training*, Pub TB-14-1, 1952, 5 cents, 4 pp

2 *Development Status of Personal Dosimeters*, Pub TB-11-4, 1952, 5 cents, 4 pp

3 *Emergency Exposures to Nuclear Radiation*, Pub TB-11-1, 1952, 5 cents, 1 p

4 *Engineering Equipment Stockpiled for Emergency Water Supply Use*, Pub TB-13-1, 1952, 5 cents, 4 pp

5 *Emergency Measurement of Radioactivity in Food and Water*, Pub TB-11-9, 1952, 5 cents, 2 pp

6 *Permissible Emergency Levels of Radioactivity in Water and Food*, Pub TB-11-8, 1952, 5 cents, 2 pp

7 *Personal Dosimeters for Radiological Defense*, Pub TB-11-2, 1952, 5 cents, 3 pp

8 *The Most Promising Personal Dosimeters for Civil Defense Use*, Pub TB-11-3, 1952, 5 cents, 4 pp

Other Publications

1 *Alert America Color Poster Series* Posters designed for official and public service civil defense exhibit and display needs to increase public understanding of the need for an Alert America Available in two sizes Large size 26 x 37 inches, each 20 cents, small size 13 x 18 inches, each 10 cents

2 *Annotated Civil Defense Bibliography for Teachers*, Pub TEB-3-2, 1951, 20 cents, 28 pp Aid for teachers in locating publications for use in civil defense planning and instruction in schools

3 *Atomic Blast Creates Fire*, Leaflet, 1951, $1 50 per 100 copies Instruction to householders on how to reduce fire hazards and prevent fires in the home

4 *Civil Defense Household First Aid Kit*, Leaflet, 1951, $1 75 per 100 Lists first aid items for a family of four or less, gives items to be stocked, quantity, substitutes, and uses

5 *Civil Defense Nursing Needs*, Pub YM-1, 1952, 15 cents, 17 pp Outlines program for increasing nursing services to insure an adequate supply of nurse power in the event of attack or disaster

6 *Interim Civil Defense Instructions for Schools and Colleges*, Pub TEB-3-1, 1951, 30 cents, 32 pp Guide for educational administrators in planning immediate civil defense training and education programs

7 *Recruiting Poster Series* 10 posters designed to assist State and local civil defense groups in the recruitment of volunteers The posters in this set depict the duties of the 10 basic civil defense services Available in two sizes Large size 26 x 37

inches, each 10 cents, set 70 cents, small size 12 x 18 inches, each 5 cents, set 20 cents

8 *The Warden's Handbook*, Pub H-7-1, 19[illegible]1, 15 cents, [illegible]1 pp Basic reference aid for the block warden

9 *What You Can Do Now!*, Leaflet, 19[illegible]3, $1.[illegible]0 per 100 copies Outlines steps for preparing the home and the family against enemy attack

10 *Women in Civil Defense*, Pub VM-2, 1952, 15 cents, 20 pp Emphasizes the importance of women's participation in the civil defense program

3 *Emergency Exposures to Nuclear Radiation*, Pub TB-11-1, 1952, 5 cents, 1 p

4 *Engineering Equipment Stockpiled for Emergency Water Supply Use*, Pub TB-13-1, 1952, 5 cents, 4 pp

5 *Emergency Measurement of Radioactivity in Food and Water*, Pub TB-11-9, 1952, 5 cents, 2 pp

6 *Permissible Emergency Levels of Radioactivity in Water and Food*, Pub TB-11-8, 1952, 5 cents, 2 pp

7 *Personal Dosimeters for Radiological Defense*, Pub TB-11-2, 1952, 5 cents 3 pp

8 *The Most Promising Personal Dosimeters for Civil Defense Use*, Pub TB-11-3, 1952, 5 cents, 4 pp

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